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L9: Entry 35 of 41 File: USPT Dec 24, 1996

DOCUMENT-IDENTIFIER: US 5587305 A
TITLE: Pasteurella haemolytica transformants

Detailed Description Text (2):
It is a discovery of the present invention that P. haemolytica contains at least one restriction-modification system, called herein the PhaI system. Both the restriction endonuclease and the methyltransferase have been molecularly cloned. One such molecular clone (E. coli PhaIMtase) has been deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md., 20852, USA, on Dec. 2, 1993, under the terms of the Budapest Treaty as Accession No. ATCC 69500. A preliminary sequence of the methyltransferase gene has been determined. The predicted amino acid sequence of the methyltransferase contains sequence motifs which are consistent with an adenine-methylating specificity.

Detailed Description Text (5):
It has also been discovered by the present inventors, that a barrier to transformation of P. haemolytica can be overcome by treating DNA with a methylating enzyme, such as the PhaI methyltransferase. Such enzymes modify DNA substrates such that endonucleases which recognize 5'-GATGC-3' or 5'-GCATC-3' sequences are inhibited in their ability to digest such modified substrates. Examples of such endonucleases are PhaI endonuclease and SfaNI endonuclease. While applicants do not wish to be bound by any particular hypothesis on the mechanism of action of such methyltransferase enzymes, it appears that the PhaI methyltransferase methylates specific adenine residues in DNA.

Detailed Description Text (51):
The possibility that a system similar to E. coli mcr, mrr, is active in P. haemolytica was investigated by passage of pPh.DELTA.roACm.sup.R pD80 through E. coli strain GM2163 previously transformed with the recombinant cosmid containing PhaI methyltransferase (Raleigh et al., Proc. Natl. Acad. Sci. 83:9070-9074 (1986)). Since strain GM2163 is dam-, the resultant DNA would only be modified at PhaI sites (Marinus et al., Mol. Gen. Genet. 192:288-289 (1983)). Efficiency of transformation with this DNA, however, was not substantially different than that using DNA obtained from PhaI Mtase which is dam-methylated (Table 1). It is possible a second restriction system, not readily detectable in cell extracts, is active in P. haemolytica A1. Genes have been described in Neisseria gonnorhea MS11 which encode for restriction enzymes which are expressed at levels too low to detect biochemically (Stein et al., J. Bact. 74:4899-4906 (1992).

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DERWENT-ACC-NO: 1998-217199
DERWENT-WEEK: 200248

TITLE: Bacterial methyl-transferase proteins - used to isolate antibiotics and inhibitors of bacterial growth

INVENTOR: BENKOVIC, S J; BERDIS, A ; KAHNG, L S ; LEE, I ; SHAPIRO, L ; STEPHENS, C ; WRIGHT, R

PATENT-ASSIGNEE: PENN STATE RES FOUND (PENNN), UNIV LELAND STANFORD JUNIOR (STRD)

PRIORITY-DATA: 1996US-020089P (September 19, 1996), 1999US-0269137 (July 19, 1999)

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PATENT-FAMILY:

	PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
☐	WO 9812206 A1	March 26, 1998	E	070	C07H021/04
☐	US 6413751 B1	July 2, 2002		000	C12N009/10
☐	AU 9744860 A	April 14, 1998		000	C07H021/04

DESIGNATED-STATES: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW AT BE CH DE DK EA ES FI FR GB GH GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
WO 9812206A1	September 17, 1997	1997WO-US16593	
US 6413751B1	September 19, 1996	1996US-020089P	Provisional
US 6413751B1	September 17, 1997	1997WO-US16593	
US 6413751B1	July 19, 1999	1999US-0269137	
US 6413751B1		WO 9812206	Based on
AU 9744860A	September 17, 1997	1997AU-0044860	
AU 9744860A		WO 9812206	Based on

INT-CL (IPC): C07 H 21/02; C07 H 21/04; C07 K 16/40; C12 N 9/10; C12 N 15/63; C12 N 15/85; C12 Q 1/48; C12 Q 1/68; G01 N 33/567

ABSTRACTED-PUB-NO: US 6413751B
BASIC-ABSTRACT:

An isolated nucleic acid encodes a methyltransferase, where the methyltransferase has a molecular

weight of about 30-45 kD and binds to a polyclonal antibody that specifically binds to a polypeptide selected from the 376, 378, 85 or 359 amino acid sequences given in the specification. Also claimed are: (1) an isolated methyltransferase protein where the methyl transferase has a molecular weight of 30-45 kD and binds to a polyclonal antibody that specifically binds to a polypeptide selected from the 376, 378, 85 or 359 amino acid sequences, and (2) an assay for methyltransferase activity comprising: (a) contacting a processive methyltransferase with a substrate selected from: 5' atcctctcgcg*a(CH3) gtcaacagaaa 3' aggagagcgc tcagttgtctttataggcgc; 5' atcctctcgcg*a(CH3) gtcaacagaaatatccgctcatcaccgcaagtt 3' aggagagcgc tcagttgtctttataggcgagtagtggcgttcaaaaggca; and 5' atcctctcgcg*a(CH3)gtcaac- agaaatatccgcgagtcaccgcaagttttccgtitgaccggc 3' aggagagcgc tcagttgtctttataggcgctcagtggcgttcaaaaggcaaactggcgtgggagg; and (b) further contacting the processive methyltransferase with a methyl donor prior to or at the same time as the addition of the DNA substrate, where the methyltransferase methylates the DNA substrate.

The nucleic acid is preferably the 1698 bp DNA sequence encoding the Rhizobium meliloti DNA methyltransferase with the 376 amino acid sequence. It can also be the 1731 bp DNA sequence encoding the 378 amino acid sequence of Brucella abortus DNA methyltransferase. The nucleic acid can be the 255 bp DNA sequence encoding the 85 amino acid sequence of Agrobacterium tumefaciens DNA methyltransferase. The nucleic acid sequence can alternatively be the 2091 bp DNA sequence of Helicobacter pylori DNA methyltransferase (all sequences given in the specification). In the method of (2), the methyl donor is S-adenosyl methionine. The assay is performed at 30 deg. C or 37 deg. C. The assay is performed in the presence of 150 mM potassium acetate.

USE - The methyltransferase proteins can be used in an assay for screening for inhibitors of DNA methyltransferase activity. They can also be used in an assay for detecting antibiotics that target processive adenine methyltransferases (both claimed). Inhibitors of the methyltransferase activity results in a migration or elimination of the subject bacteria to infect and/or grow and/or proliferate in an animal or plant host.

ABSTRACTED-PUB-NO: WO 9812206A
EQUIVALENT-ABSTRACTS:

An isolated nucleic acid encodes a methyltransferase, where the methyltransferase has a molecular weight of about 30-45 kD and binds to a polyclonal antibody that specifically binds to a polypeptide selected from the 376, 378, 85 or 359 amino acid sequences given in the specification. Also claimed are: (1) an isolated methyltransferase protein where the methyl transferase has a molecular weight of 30-45 kD and binds to a polyclonal antibody that specifically binds to a polypeptide selected from the 376, 378, 85 or 359 amino acid sequences, and (2) an assay for methyltransferase activity comprising: (a) contacting a processive methyltransferase with a substrate selected from: 5' atcctctcgcg*a(CH3) gtcaacagaaa 3' aggagagcgc tcagttgtctttataggcgc; 5' atcctctcgcg*a(CH3) gtcaacagaaatatccgctcatcaccgcaagtt 3' aggagagcgc tcagttgtctttataggcgagtagtggcgttcaaaaggca; and 5' atcctctcgcg*a(CH3)gtcaac- agaaatatccgcgagtcaccgcaagttttccgtitgaccggc 3' aggagagcgc tcagttgtctttataggcgctcagtggcgttcaaaaggcaaactggcgtgggagg; and (b) further contacting the processive methyltransferase with a methyl donor prior to or at the same time as the addition of the DNA substrate, where the methyltransferase methylates the DNA substrate.

The nucleic acid is preferably the 1698 bp DNA sequence encoding the Rhizobium meliloti DNA methyltransferase with the 376 amino acid sequence. It can also be the 1731 bp DNA sequence encoding the 378 amino acid sequence of Brucella abortus DNA methyltransferase. The nucleic acid can be the 255 bp DNA sequence encoding the 85 amino acid sequence of Agrobacterium tumefaciens DNA methyltransferase. The nucleic acid sequence can alternatively be the 2091 bp DNA sequence of Helicobacter pylori DNA methyltransferase (all sequences given in the specification). In the method of

(2), the methyl donor is S-adenosyl methionine. The assay is performed at 30 deg. C or 37 deg. C. The assay is performed in the presence of 150 mM potassium acetate.

USE - The methyltransferase proteins can be used in an assay for screening for inhibitors of DNA methyltransferase activity. They can also be used in an assay for detecting antibiotics that target processive adenine methyltransferases (both claimed). Inhibitors of the methyltransferase activity results in a migration or elimination of the subject bacteria to infect and/or grow and/or proliferate in an animal or plant host.

CHOSEN-DRAWING: Dwg.0/8

DERWENT-CLASS: B04 D16 S03
CPI-CODES: B04-E02F; B04-N03; B11-C08E3; B12-K04; D05-H09; D05-H12A;
EPI-CODES: S03-E14H4;

8/9/69
DIALOG(R)File 155:MEDLINE(R)

08326251 PMID: 2510127
Single amino acid changes that alter **the DNA sequence specificity of the DNA-[N6-** adenine **]** methyltransferase **(Dam) of bacteriophage T4.**
Miner Z; Schlagman S L; Hattman S
Department of Biology, University of Rochester, NY 14627.
Nucleic acids research (ENGLAND) Oct 25 1989, 17 (20) p8149-57, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: GM29227; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Bacteriophage T4 codes for a DNA-[N6- **adenine**] **methyltransferase** (Dam) which recognizes primarily the sequence GATC in both cytosine- and hydroxymethylcytosine-containing DNA. Hypermethylating **mutants** , damh, exhibit a relaxation in sequence specificity, that is, they are readily able to methylate non-canonical sites. We have determined that the damh **mutation** produces a single amino acid change (Pro126 to Ser126) in a region of homology (III) shared by three DNA- **adenine methyltransferases** ; viz, T4 Dam, Escherichia coli Dam, and the DpnII modification enzyme of Streptococcus pneumoniae. We also describe another **mutant** , damc, which methylates GATC in cytosine-containing DNA, but not in hydroxymethylcytosine-containing DNA. This **mutation** also **alters** a single amino acid (Phe127 to Val127). These results implicate homology region III as a domain involved in DNA sequence recognition. The effect of several different amino acids at residue 126 was examined by creating a polypeptide chain terminating codon at that position and comparing the methylation capability of partially purified enzymes produced in the presence of various suppressors. No enzyme activity is detected when phenylalanine, glutamic acid, or histidine is **inserted** at position 126. However, **insertion** of alanine, cysteine, or glycine at residue 126 produces enzymatic activity similar to Damh.
Tags: Support, U.S. Gov't, P.H.S.
Descriptors: DNA, Viral--genetics--GE; *Escherichia coli--genetics--GE; *Genes, Structural, Viral; * **Mutation** ; *Proline; *Serine; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; *T-Phages --genetics--GE; Amino Acid Sequence; Base Sequence; Codon--genetics--GE; Escherichia coli--enzymology--EN; Kinetics; Molecular Sequence Data; **Plasmids** ; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific) --isolation and purification--IP; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME; T-Phages--enzymology--EN
CAS Registry No.: 0 (Codon); 0 (DNA, Viral); 0 (Plasmids); 147-85-3 (Proline); 56-45-1 (Serine)
Enzyme No.: EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19891204
Record Date Completed: 19891204

8/9/70
DIALOG(R)File 155:MEDLINE(R)

08253594 PMID: 2549371
The Escherichia coli dam gene is expressed as a distal gene of a new operon.
Jonczyk P; Hines R; Smith D W
Department of Biology, University of California, San Diego, La Jolla 92093.
Molecular & general genetics - MGG (GERMANY, WEST) May 1989, 217 (1)

constitutively suggested that translation of the second leader peptide is controlled by ribosome stalling in the first leader peptide. From Northern RNA blot analysis of ermD transcription, it appears that regulation of ermD expression is not by transcriptional **attenuation** .

Tags: Comparative Study; Support, U.S. Gov't, P.H.S.

Descriptors: *Anti-Bacterial Agents--pharmacology--PD; *Bacillus subtilis --genetics--GE; *Gene Expression Regulation, Bacterial; *Methyltransferases --genetics--GE; *Virginiamycin--pharmacology--PD; Amino Acid Sequence; Base Sequence; Codon; DNA **Mutational** Analysis; Drug Resistance, Microbial --genetics--GE; Erythromycin--pharmacology--PD; Molecular Sequence Data; Nucleic Acid Conformation; Protein Sorting Signals--genetics--GE; Recombinant Fusion Proteins--genetics--GE; Regulatory Sequences, Nucleic Acid--genetics--GE; Transcription, Genetic; Translation, Genetic

CAS Registry No.: 0 (Anti-Bacterial Agents); 0 (Codon); 0 (Protein Sorting Signals); 0 (Recombinant Fusion Proteins); 11006-76-1 (Virginiamycin); 114-07-8 (Erythromycin)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.66 (rRNA (**adenosine** -O-2'-) **methyltransferase**)

Gene Symbol: ermD

Record Date Created: 19921013

Record Date Completed: 19921013

8/9/52

DIALOG(R)File 155:MEDLINE(R)

09387306 PMID: 1641327

Expression of Escherichia coli dam gene in Bacillus subtilis provokes DNA damage response: N6-methyladenine is removed by two repair pathways.

Guha S; Guschlbauer W

Departement de Biologie Cellulaire et Moleculaire, Centre d'Etudes de Saclay, Gif-sur-Yvette, France.

Nucleic acids research (ENGLAND) Jul 25 1992, 20 (14) p3607-15, ISSN 0305-1048 Journal Code: 0411011

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The dam gene of Escherichia coli encodes a DNA methyltransferase that methylates the N6 position of adenine in the sequence GATC. It was stably expressed from a shuttle **vector** in a repair- and recombination-proficient strain of Bacillus subtilis. In this strain the majority of **plasmid** DNA molecules was modified at dam sites whereas most chromosomal DNA remained unmethylated during exponential growth. During stationary phase the amount of unmethylated DNA increased, suggesting that methylated bases were being removed. An ultraviolet damage repair-deficient **mutant** (uvrB) contained highly methylated chromosomal and **plasmid** DNA. High levels of Dam methylation were detrimental to growth and viability of this **mutant** strain and some features of the SOS response were also induced. A **mutant** defective in the synthesis of adaptive DNA alkyltransferases and induction of the adaptive response (ada) also showed high methylation and properties similar to that of the dam gene expressing uvrB strain. When protein extracts from B. subtilis expressing the Dam methyltransferase or treated with N-methyl-N'-nitro-N-nitroso-guanidine were incubated with [3H]-labelled Dam methylated DNA, the methyl label was bound to two proteins of 14 and 9 kD. Some free N6-methyladenine was also detected in the supernatant of the incubation mixture. We propose that N6-methyladenine residues are excised by proteins involved in both excision (uvrB) and the adaptive response (ada) DNA repair pathways in B. subtilis.

Tags: Support, Non-U.S. Gov't

Descriptors: Adenine--analogs and derivatives--AA; *Bacillus subtilis --genetics--GE; *DNA Repair--genetics--GE; *Escherichia coli--enzymology --EN; *Methyltransferases--genetics--GE; *Site-Specific DNA- **Methyltransfer ase** (**Adenine** -Specific); Adenine--metabolism--ME; Bacillus subtilis--drug effects--DE; Bacillus subtilis--radiation effects--RE; Chromatography,

Briggs et al., "Characterization of a Restriction Endonuclease, PhaI, from Pasteurella haemolytica Serotype A1 and Protection of Heterologous DNA by a Cloned PhaI Methyltransferase Gene", Applied and Environmental Microbiology 60(6):2006-2010 (1994).

WEST Search History

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DATE: Monday, November 01, 2004

Hide?	Set Name	Query	Hit Count
	DB=USPT; PLUR=YES; OP=AND		
☐	L1	dam.ti,ab,clm.	4138
☐	L2	L1 and (adenine or adenosine)	17
☐	L3	(adenine or adenosine).clm. same ($methylase or $transferase).clm.	75
☐	L4	L3 not l2	75
☐	L5	(adenine or adenosine).clm. same ($methylase or $methyltransferase or $methyl-transferase).clm.	7
☐	L6	dam near5 (mutation or mutant or mutagenesis or altered or alteration or modified or modification or insertion or deletion or substitution or inserted)	468
☐	L7	L6 and (adenine or adenosine)	42
☐	L8	l7 not l7 not l5	0
☐	L9	l7 not l4 not l5	41
☐	L10	deoxyadenosine near2 methyl near2 transferase	1
☐	L11	dna near2 methyl near2 transferase	46
☐	L12	dna near2 methyl near transferase	46
☐	L13	dna near methyl near transferase	40
☐	L14	dna near methyltransferase	238
☐	L15	dna near2 methyltransferase	266
☐	L16	dnamethyltransferase	0
☐	L17	(L16 or l15 or l14 or l13 or l12 or l11)	280
☐	L18	L17 same (adenine or adenosine)	34
☐	L19	L17 and (adenine or adenosine)	159
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND		
☐	L20	deoxyadenosine near2 methyl near2 transferase	1
☐	L21	dna near2 methyl near2 transferase	121
☐	L22	dna near2 methyl near transferase	121
☐	L23	dna near methyltransferase	633
☐	L24	dnamethyltransferase	1
☐	L25	(L24 or l23 or l22 or l21 or l20)	681
☐	L26	L25 same (adenine or adenosine or gatc)	74

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 50 of 74 returned.

☐ 1. 20040209257. 16 Apr 03. 21 Oct 04. Method for cloning and expression of AcuI restriction endonuclease and AcuI methylase in E. coli. Samuelson, James, et al. 435/6; 435/199 435/252.3 435/320.1 435/69.1 536/23.2 C12Q001/68 C07H021/04 C12N009/22 C12N015/74.

☐ 2. 20040201117. 23 Jul 03. 14 Oct 04. COATED PARTICLES, METHODS OF MAKING AND USING. Anderson, David. 264/4.3; 264/4.6 428/402.2 428/402.21 B01J013/02.

☐ 3. 20040175816. 03 Mar 03. 09 Sep 04. Method for cloning and expression of OkrAI restriction endonuclease and methyltransferase. Xu, Shuang-Yong, et al. 435/199; 435/252.3 435/320.1 435/69.1 536/23.2 C12N009/22 C07H021/04 C12N001/21 C12N015/74.

☐ 4. 20040132048. 26 Jun 03. 08 Jul 04. Methods and compositions for determining methylation profiles. Martienssen, Robert, et al. 435/6; C12Q001/68.

☐ 5. 20040091911. 10 Jul 03. 13 May 04. Recombinant type II restriction endonucleases, MmeI and related endonucleases and methods for producing the same. Morgan, Richard D., et al. 435/6; 435/199 435/252.3 435/320.1 435/69.1 536/23.2 C12Q001/68 C07H021/04 C12N009/22 C12N001/21 C12N015/74.

☐ 6. 20040063126. 14 Aug 03. 01 Apr 04. Electrochemical sensor using intercalative, redox-active moieties. Barton, Jacqueline K., et al. 435/6; C12Q001/68.

☐ 7. 20040052840. 23 Jun 03. 18 Mar 04. Preparations for oligonucleotide transfer. Kubota, Shunichiro, et al. 424/468; 514/44 A61K009/22 A61K048/00.

☐ 8. 20040018522. 06 May 03. 29 Jan 04. Identification of dysregulated genes in patients with multiple sclerosis. Dangond, Fernando, et al. 435/6; 702/20 C12Q001/68 G06F019/00 G01N033/48 G01N033/50.

☐ 9. 20030232340. 13 Jun 02. 18 Dec 03. Nanoporous particle with a retained target. Anderson, David. 435/6; 435/287.2 435/7.1 C12Q001/68 G01N033/53 C12M001/34.

☐ 10. 20030212455. 11 Mar 03. 13 Nov 03. Identification of in vivo dna binding loci of chromatin proteins using a tethered nucleotide modification enzyme. Van Steensel, Bas, et al. 623/6.6; 424/427 435/6 A61F002/16 C12Q001/68 A61F002/00.

☐ 11. 20030166206. 26 Feb 02. 04 Sep 03. METHOD FOR CLONING AND EXPRESSION OF MSPA1I RESTRICTION ENDONUCLEASE AND MSPA1I METHYLASE IN E. COLI. Xu, Shuang-Yong, et al. 435/193; 435/199 435/320.1 435/325 435/69.1 536/23.2 C07H021/04 C12N009/10 C12N009/22 C12P021/02 C12N005/06.

☐ 12. 20030130797. 17 Oct 01. 10 Jul 03. Protein modeling tools. Skolnick, Jeffrey, et al. 702/19; G06F019/00 G01N033/48 G01N033/50.

☐ 13. 20030114402. 07 Dec 01. 19 Jun 03. Modulators of DNA cytosine-5 methyltransferase and methods for use thereof. Reich, Norbert O., et al. 514/44; 536/23.1 A61K048/00 C07H021/04.

L9: Entry 4 of 41 File: USPT Mar 30, 2004

DOCUMENT-IDENTIFIER: US 6713285 B2
TITLE: Circular site-directed mutagenesis

Brief Summary Text (13):
The template digesting step in the methods of the invention may be carried out in any of a variety of methods involving a selection enzyme. The selection enzyme, e.g., a restriction endonuclease, is an enzyme that digests parental polynucleotides and does not digest newly synthesized mutagenized polynucleotides. Either template polynucleotides prior to replication are modified or polynucleotides synthesized during replication are modified so that the selection enzyme preferentially catalyzes the digestion of the parent template polynucleotide. In one embodiment of the invention the polynucleotide for mutagenesis is dam methylated double-stranded DNA and the restriction enzyme used to to digest parental polynucleotide strands is Dpn I.

Detailed Description Text (18):
The modifying step for use in conjunction with a parental strand digestion step may comprise the process of exposing a DNA molecule for modification to a modifying agent. The modification step may be carried out before the linear cyclic amplification reaction step or during the linear cyclic amplification reaction step. The modifying agent may be a methylase enzyme that catalyzes the methylation of a base within the polynucleotide of interest. Examples of suitable methylases for use in the invention include dam methylase, dcm methylase, Alu I methylase, and the like. The modification reaction may take place in vivo or in vitro. In vivo methylation may be conveniently achieved by propagating polynucleotides in cells, either prokaryotic or eukaryotic, that endogenously produce a suitable methylase enzyme. In a preferred embodiment of the invention, in vivo methylation is used to carry out the modification step. The polynucleotide modification step may also be accomplished by synthesizing polynucleotides with nucleotides comprising a modified base, e.g., 6-methyl-ATP, rather than directly modifying a polynucleotide after the polynucleotide has been completely synthesized. When the modification reaction is a methylation reaction and the selection enzyme is a restriction endonuclease that requires methylated bases for activity, the methylation step is preferably performed in vivo. When the selection enzyme is a restriction endonuclease that does not cleave its recognition sequence when the recognition sequence of the enzyme is unmethylated, the modification reaction is preferably a methylation reaction performed in vitro by a polymerase catalyzing the incorporation of methylated nucleotides into a newly synthesized polynucleotide strand. When the selection enzyme used in the digestion step is Dpn I, the modification step is preferably the methylation of adenine to produce 6-methyl adenine (dam methylase) and the methylation reaction preferably takes place in vivo by propagating the DNA for mutagenesis as a plasmid in a suitable prokaryotic host cell.

Detailed Description Text (20):
Restriction endonucleases are preferred for use as selection enzymes in the digestion step. A preferred selection enzyme for use in the parental strand digestion step is the restriction endonuclease Dpn I, which cleaves the polynucleotide sequence GATC only when the adenine is methylated (6-methyl adenine). Other restriction endonucleases suitable for use in the parental strand

digestion step include Nan II, NmuD I, and NmuE I. However, restriction endonucleases for use as selection enzymes in the digestion step do not need to be isoschizomers of Dpn I.

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L9: Entry 8 of 41 File: USPT Jun 18, 2002

DOCUMENT-IDENTIFIER: US 6406896 B1
TITLE: Transposase enzyme and method for use

Drawing Description Text (5):
FIG. 4 depicts the relative preference of mutant transposases obtained in successive rounds of mutagenesis/recombination for OE and IE in a dam- strain of E. coli.

Detailed Description Text (7):
In the first method, the mutant IE end binding sequence contains an adenine in place of thymine at position 12 ("IE12A"; SEQ ID NO:5). The thymine-to-adenine change in IE12A destroys one of the two methylation sites of wild-type IE. Applicants used sPCR, a combinatorial, random directed mutagenesis technique to obtain modified transposase proteins that can restore transposition activity to polynucleotides flanked with IE12A. sPCR was developed and described gtemmer, W. P., "Rapid evolution of a protein in vitro by DNA shuffling," Nature 370:389-391 (1994) and Stemmer, W. P., "DNA shuffling by random fragmentation and reassembly: in vitro recombination for molecular evolution," Proc. Natl. Acad. Sci. U.S.A. 91:10747-10751 (1994), both of which are incorporated by reference herein as if set forth in their entirety. Briefly, DNA is manipulated in vitro in the SPCR method to introduce point mutations and to allow random recombination within a population of mutant sequences. The mutated genes can be cloned into plasmids which can be selected for increased activity in vivo. Clones having desirable phenotypes are then used as substrates for subsequent rounds of mutagenesis/recombination and selection for a further improved phenotype.

Detailed Description Text (34):
Recently a protein-DNA co-crystal representing Tnp EK/LP complexed with pre-cut (no donor backbone) OE DNA has been solved. In this complex amino acid 58 is shown to interact specifically with OE at position 10. This places the mutant residue in the vicinity of position 12, the nucleotide mutation that it was initially chosen to repress. Positions 10, 11, and 12 are all different between IE and OE and furthermore it is near one of two areas that contain major groove modification by dam methylase (position 11 of top strand and position 12 of bottom strand).

Detailed Description Text (39):
The inside end of the Tn5 transposon contains two GATC signal sequences that add four methyl groups into the major groove of each end. In this study, we were able to isolate a single mutation, E58V, which not only overcomes this binding inhibition but also appears to preferentially function on transposons in which the methyl groups are present (presumably due to increased binding affinity). Furthermore, the co-crystal structure of Tnp EK/LP complexed with pre-cleaved DNA shows that glutamate 58 interacts directly with position 10 of OE in the major groove. This region is in the vicinity of the methyl group that is present on the adenine of the non-transferred strand of IE.sup.ME. The fact that a single amino acid change can result in this extreme change in phenotype leads us to propose that this methyl group alone is responsible for the inhibition of binding of transposase to IE.sup.ME. The inhibition of binding by Tnp WT to IE.sup.ME is likely caused by an interaction between the methyl group and the negatively charged side chain of

glutamate 58. Replacing the residue with a valine can not only remove an unfavorable unfavorable interaction but also lead to an increase in binding affinity due to hydrophobic packaging between the side chain of the valine residue and this methyl group.

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L9: Entry 15 of 41 File: USPT Jan 2, 2001

DOCUMENT-IDENTIFIER: US 6168918 B1
**** See image for Certificate of Correction ****
TITLE: Method of detecting foreign DNA integrated in eukaryotic chromosomes

Detailed Description Text (3):
As used herein, "dam" is meant to refer to the enzyme deoxyadenosine methyl transferase. The enzyme dam methylates the adenine when it occurs in the nucleotide sequence GATC.

Detailed Description Text (4):
As used herein, "dam.sup.+ cells" is meant to refer to cells that produce dam. Certain bacterial strains such as E. coli K12 strains produce dam naturally. Bacterial strains as well as eukaryotic host cells such as yeast, insect cells, mammalian cells, plant cells, etc. may also be transformed with an expression vector that encodes dam and such cells are converted into dam.sup.+ cells. DNA that contains the sequence GATC and that is produced in dam.sup.+ cells will have the adenine in the GATC sequence methylated.

Detailed Description Text (6):
As used herein, "dam.sup.- cells" is meant to refer to cells which do not have dam. Unless transformed within heterologous dam gene, eukaryotic cells do not contain dam and are therefore dam.sup.- cells. DNA that contains the sequence GATC that is produced or repaired in dam.sup.- cells will not have the adenine methylated.

Detailed Description Text (12):
DpnI is a restriction enzyme described in U.S. Pat. No. 4,960,707 issued Oct. 2, 1990 to Lacks, which is incorporated herein by reference. It is known that the restriction enzyme DpnI recognizes and cleaves nucleic acid molecules at the nucleotide sequence GATC if the adenine is methylated. DpnI will not recognize and cleave nucleic acid molecules at the nucleotide sequence GATC if the adenine is not methylated.

Detailed Description Text (14):
The invention relates to a PCR method which utilizes the selective digestion of certain DNA molecules by DpnI. DpnI will only digest DNA having a specific sequence and methylation pattern. The endonuclease DpnI digests potential "contaminant" free plasmid DNA molecules before the PCR reaction is carried out. The DpnI restriction endonuclease recognizes and cleaves GATC sequences that are methylated at the adenine residue, but does not cleave unmethylated GATC sequences. Since DpnI will only cleave DNA molecules at the GATC sequence if the adenine is methylated, DpnI will cleave plasmid DNA molecules that have the sequence GATC if the plasmid DNA is produced in dam.sup.+ cells. DpnI will not cleave eukaryotic chromosomal DNA molecules if the eukaryotic cells are dam.sup.-. Furthermore, DpnI will not cleave plasmid DNA integrated into eukaryotic chromosomal DNA if the integrated plasmid DNA has been repaired or reproduced in the eukaryotic cell. Plasmid DNA that has been integrated into eukaryotic chromosomal DNA (or any other dam.sup.- cell) and which has undergone subsequent repair and/or replication, will acquire the unmethylated GATC pattern.

Detailed Description Text (16):
The eukaryotic cells used in the invention are dam.sup.- that their chromosomal DNA molecules do not have the characteristic methylation pattern associated with dam.sup.+ cells. That is, in each instance in which the chromosomal DNA molecule of a dam.sub.- cell has the sequence GATC, the adenine is not methylated. Therefore, the chromosomal DNA molecule of a dam.sub.- cell will not be cut by DpnI at that site. In preferred embodiments, the eukaryotic cells that are used are from test animals which have been administered plasmid DNA molecules. In some embodiments, animals are administered plasmid DNA and total DNA is extracted from samples of cells and/or tissue at and/or near the site where the plasmid DNA was administered. In some embodiments, the animal is a mammal such as a monkey, a dog, a rabbit or a rodent, particularly a rat or mouse. In some embodiments, the animal is a human or non-human primate.

Detailed Description Text (17):
The plasmid DNA molecules used in the invention are produced in dam.sup.+ cells. Accordingly, in each instance in which the plasmid DNA molecule has the sequence GATC, the adenine is methylated. In some preferred embodiments, the dam.sup.+ cells are dam.sup.+ bacterial cells. In some preferred embodiments, the dam.sup.+ cells are dam.sup.+ E. coli cells. In some preferred embodiments, the dam.sup.+ cells are dam.sup.+ E. coli K12 strains, e.g., DH10B, W3110, EMG2, and DH5.alpha.. Although the dam enzyme occurs naturally in a number of bacteria, especially E. coli K12 strains, dam can be cloned into bacteria, yeast, plants, insect cells or even mammalian cells, so that the described integration assay can be utilized using vector DNA molecules produced in such dam.sup.+ systems. Alternatively, the plasmid can be methylated using isolated dam enzyme in vitro.

Detailed Description Text (25):
To eliminate the DNA digestion segments produced by digestion of free plasmid DNA molecules, each fraction is digested with DpnI before carrying out PCR. The DpnI digests all DNA that exhibits dam.sup.+ methylation at the sequence GATC but will not digest the GATC sequence with an unmethylated adenine. Thus, free plasmid DNA will be digested at a site within the sequence between the sequences to which the plasmid-specific primers hybridize. DpnI digestion is done under optimal conditions in PCR buffer which has been modified to contain 5 mM DTT protocols using manufacturers instructions.

Detailed Description Text (26):
Following DpnI digestion, fragments of DNA are amplified with plasmid-specific primers which flank DpnI sites. Only uncut DNA will yield amplification products. Thus, only fragments of integrated plasmid DNA which has undergone repair or replication will be amplified. DNA replication and/or DNA repair following integration converts the integrated sequence to a mammalian pattern. The adenine of GATC is no longer methylated. DpnI will no longer cut it, and it will be amplified by PCR. Primer sequences are selected based upon their being adjacent to a DpnI site in the plasmid DNA sequence. Primers can be 10-200 nucleotides in length, more preferably 30 nucleotides in length to amplify a sequence of 20-200 nucleotides which includes a DpnI site between the sequences that hybridize to the primers. The sequence between the primers will preferably contain more than one DpnI site, more preferably, three or more DpnI sites. A plurality of DpnI sites is preferable because some GATC sites in the plasmid may escape methylation even in dam.sup.+ bacteria. Sensitive PCR can be achieved with DNA segments of 10 Kb or even greater, but it is preferable that the segment to be amplified is less than 1 Kb. Even smaller segments (e.g., 100 bases), can be amplified, but with smaller segments, methods for detection of amplified fragments might have to be modified, e.g., use of a higher gel, OT HPLC or by incorporation of radiolabelled into product. Amplification is done following standard protocols using manufacturers instructions. In some preferred embodiments, sets of primers amplify fragments, which include at least one DpnI site between primer sequences, of up to 10000 nucleotides. In some preferred embodiments, sets of primers amplify fragments 25-

5000 nucleotides. In some preferred embodiments, sets of primers amplify fragments 1000-2000 nucleotides. In some preferred embodiments, sets of primers amplify fragments 500-1500 nucleotides. In some preferred embodiments, sets of primers amplify fragments 1000-4000 nucleotides. In some preferred embodiments, sets of primers amplify fragments about 100-500 nucleotides.

Detailed Description Text (41):
Choice of primer positions in DpnI-PCR is also selected in some embodiments as a means to increase performance of the assay. The primer positions have been chosen to span at least 3 DpnI sites. The reason for this is as follows. Dam methylase methylates at the N.sup.6 position of adenines in a GATC sequence. However, in E. coli, KK strains containing a single copy of a wild-type dam methylase, a particular GATC sequence is methylated only in .about.99% of the plasmid molecules. Therefore, if a primer was designed to span only one DpnI site, nearly 1% of the plasmid molecules would escape cleavage of DpnI and, therefore, be detectable by PCR and contribute to false positives. It was experimentally determined that PCR primers must span at least 3 GATC sites to ensure that at least 1 GATC site in 10.sup.8 plasmid molecules was methylated at the N.sup.6 of adenines, and therefore be cleaved by DpnI. However, in strains containing either cloned copies of Dam genes, or with modified Dam methylase wherein the methylation of GATC sequence is nearly 10.sup.8 out of 10.sup.8 sites, primers may be designed to span just one GATC sequence.

Detailed Description Text (43):
According to the present invention, the DNA from the sample is subjected to digestion by DpnI. All of the plasmid DNA not integrated into the eukaryotic chromosome is recognized and digested. However, no eukaryotic chromosomal DNA is digested. Nor is plasmid DNA integrated into the eukaryotic chromosomal DNA that has undergone repair or replication, processes known to alter the GATC adenine methylation pattern. Thus, the assay can be used to detect the presence of plasmid DNA integrated into the eukaryotic chromosomal DNA that has undergone repair or replication by selectively digesting non-integrated, non-repaired or non-replicated DNA.

Detailed Description Text (53):
To solve this problem, it was necessary to take advantage of a chemical difference between mammalian genomic DNA and E. coli (dam+) produced plasmid DNA. The deoxyadenosine methylase (Dam) of E. coli K12 strains is an unique enzyme that methylates adenine nucleotides when present as a GATC sequence. GATC methylation at the adenines is signatory to E. coli K12 DNA. Mammalian cells do not have a GATC sequence-dependent deoxyadenosine methylase activity. Furthermore, the restriction endonuclease DpnI is known to only recognize and cleave at GATC sequences with the E. coli K12 methylation pattern. Pre-treatment of DNA isolated from tissues with DpnI therefore effectively eliminates free plasmid sequences within a mixture of free plasmid and chromosome-integrated plasmid sequences. Upon elimination of free plasmid DNA in the mixture, it is possible to use the specificity and sensitivity of the PCR to analyze for plasmid DNA integrated at random sites within the chromosome.

Detailed Description Text (55):
DpnI PCR is quite simple. After chromosomal DNA is prepared, by any standard method, it is added to a standard PCR reaction mixture, with thermostable polymerase, primers, etc. At this point, DpnI (1-2 .mu.L, 20-40 units) is also added to the PCR mixture. The tubes are closed (sealed) and the standard PCR is modified by programming two pre-incubation steps prior to standard thermocycling. The first step is at elevated temperature (60.degree. C., 10 min) which removes contaminating nuclease activity in DpnI preps by heat inactivation. This step does not destroy DpnI activity. This step could be eliminated if phosphorothioate primers are used in the PCR. The second pre-incubation step (37.degree. C., 60 min) allows for complete digestion and removal of adenine-methylated plasmid DNA.

Standard PCR thermocycling is then carried out (on the same sealed tubes) for signal signal amplification. The signal in this case would be generated specifically from integrated plasmid DNA sequences, and not from free (non-integrated) plasmid.

Detailed Description Text (60):
A known amount of positive control DNA is mixed with known amounts of genomic DNA, prior to digestion by restriction enzymes other than DpnI. The positive control is the plasmid sequence not methylated on the adenines in the GATC sites, so that the plasmid sequence is not cleaved in the subsequent digestion step by DpnI in the DpnI-PCR. Non-methylated adenines in the GATC sites in the plasmid sequence can either be prepared by isolating plasmid DNA from dam.sup.- mutants of E. coli, eg. JM110.

Detailed Description Text (62):
The unmethylated form of DNA (at the adenines in the GATC sites) can also be prepared by selecting a stably integrated cell line that contains a known defined number of plasmid integrants. An RD cell line (human rhabdomyosarcoma cell line), that was transfected with the plasmid which was previously engineered to contain a selectable marker such as an antibiotic resistance gene (hygromycin) has been used for this purpose. The cells were allowed to grow in the presence of hygromycin following transfection. When the cells reached confluency, single colonies were isolated by limiting dilution. These single colonies were built up to larger flask cultures, and the DNA was isolated. The DNA was characterized to contain the plasmid sequence by PCR using hygromycin specific primers. In order to select a cell line that contained a singular whole plasmid integrant, the DNA was digested with a set of restriction enzymes either singly or in combination, either using enzymes that digest within the plasmid of exclusively outside of the plasmid. The pattern of fragments detected in an autoradiogram when probed with labelled plasmid DNA will aid in the selection of a singular whole plasmid integrated cell line.

Detailed Description Text (94):
The effect of DpnI on Adenine Methylated and Unmethylated GATC Containing Sequences was evaluated. Rabbit genomic DNA (0.2 .mu.g), 95-03 plasmid from E. coli strain DH10B (0.2 .mu.g), 95-03 plasmid from E. coli strain JM110 (dam.sup.-, 0.2 .mu.g), rabbit genomic DNA (0.2 .mu.g) pre-incubated at 37.degree. C. overnight in the presence of 40 units of DpnI, 95-03 plasmid from E. coli strain DH10B (0.2 .mu.g) pre-incubated at 37.degree. C. overnight in the presence of 40 units of DpnI, 95-03 plasmid from E. coli strain JM110 (dam.sup.-, 0.2 .mu.g) pre-incubated at 37.degree. C. overnight in the presence of 40 units of DpnI, rabbit genomic DNA (0.2 .mu.g) digested overnight with 10 units of BclI were compared by gel electrophoresis. DpnI does not digest chromosomal DNA, despite several hours of over-digestion with four times more DpnI than recommended by the manufacturer. Similarly, DpnI did not digest non-methylated plasmid sequences when derived from the dam strain of E. coli, JM110.

Detailed Description Text (95):
To demonstrate that adenine in GATC Sequences is not methylated in eukaryotic DNA, DpnII was used. 2 .mu.g of rabbit genomic DNA digested with 20 units of DpnII and compared by electrophoresis to 2 .mu.g rabbit genomic DNA incubated with 20 units of DpnI. Digestions were carried out overnight at 37.degree. C. Results demonstrate that adenines in genomic DNA are not methylated at GATC sequences as evidenced by the DpnII mediated digestion of rabbit skeletal muscle DNA. DpnII cleaves only GATC sequences when they are methylated.

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L10: Entry 1 of 1 File: USPT Jan 2, 2001

DOCUMENT-IDENTIFIER: US 6168918 B1

**** See image for Certificate of Correction ****

TITLE: Method of detecting foreign DNA integrated in eukaryotic chromosomes

Detailed Description Text (3):

As used herein, "dam" is meant to refer to the enzyme deoxyadenosine methyl transferase. The enzyme dam methylates the adenine when it occurs in the nucleotide sequence GATC.

Previous Doc Next Doc Go to Doc#

File 155:MEDLINE(R) 1951-2004/Oct W4
(c) format only 2004 The Dialog Corp.

Set	Items	Description

Cost is in DialUnits
?ds

Set	Items	Description
S1	197749	ADENOSINE? OR ADENINE?
S2	15625	METHYLTRANSFERASE?
S3	1196	S1 (3N) S2
S4	462	S3 AND (NEGATIVE OR MUTANT? OR MUTATION? OR MUTAGENE? OR ALTER? OR REDUC? OR ATTENUAT? OR AVIRUL? OR LOSS?)
S5	177	S4/1999:2004
S6	285	S4 NOT S5
S7	89	S6 AND (HETEROLOG? OR FOREIGN? OR PLASMID? OR VECTOR? OR TRANSFORM? OR INSERT?)
S8	98	S6 AND (HETEROLOG? OR FOREIGN? OR PLASMID? OR VECTOR? OR TRANSFORM? OR INSERT? OR CARR?)
S9	50	TARGET - S8

?s s8 and (immune? or antibod? or igg or igm or iga or serum or sera or antiser? or immunoglob? or globulin? or polyclonal? or monoclonal?)

	98	S8
	259801	IMMUNE?
	671378	ANTIBOD?
	80430	IGG
	41183	IGM
	31142	IGA
	546818	SERUM
	114904	SERA
	55723	ANTISER?
	216829	IMMUNOGLOB?
	53121	GLOBULIN?
	36265	POLYCLONAL?
	177931	MONOCLONAL?
S10	0	S8 AND (IMMUNE? OR ANTIBOD? OR IGG OR IGM OR IGA OR SERUM OR SERA OR ANTISER? OR IMMUNOGLOB? OR GLOBULIN? OR POLYCLONAL? OR MONOCLONAL?)

?t s8/9/all

8/9/1
DIALOG(R)File 155:MEDLINE(R)

14028863 PMID: 9728794

Possible rare involvement of O6-methylguanine formation as a significant mutational **factor in mouse urinary bladder carcinogenesis models.**

Chen T; Yamamoto S; Kitano M; Murai T; Wanibuchi H; Matsukuma S; Nakatsuru Y; Ishikawa T; Fukushima S

First Department of Pathology, Osaka City University Medical School, Osaka, Japan.

Teratogenesis, carcinogenesis, and mutagenesis (UNITED STATES) 1998, 18 (3) p101-10, ISSN 0270-3211 Journal Code: 8100917

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

O6-methylguanine is known as one of the major premutagenic lesions in the human and rodent carcinogenesis process. O6-methylguanine-DNA methyltransferase (MGMT), which repairs methylated guanine bases, might prevent the G:C to A:T transition, and transgenic mice **carrying** this MGMT gene have been reported to be less sensitive to the carcinogenicity of certain alkylating agents. Here we utilized MGMT transgenic mice to assess the significance of O6-methylguanine formation during urinary bladder carcinogenesis. In experiment 1, 100 and 60 ppm

N-butyl-N(4-hydroxybutyl)nitrosamine was given for 20 weeks to transgenic and non-transgenic mice in their drinking water. The incidences of urinary bladder carcinomas were not different between transgenic mice and non-transgenic mice. The **mutational** spectrum of the p53 gene was evaluated by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis and direct sequencing. The pattern of p53 **mutations** of transgenic and non-transgenic mice did not differ, and the frequencies of **mutations** were 40% and 42%, respectively. G:C to A:T transition **mutations** were particularly infrequent (1 of 14 **mutations** , 7%). In experiment 2, N-methyl-N-nitrosourea, which might induce O6-methylguanine in affected alleles, was given once a week, 3 times (total 5 mg) by direct instillation into the urinary bladder through an abdominal incision. No significant neoplastic lesions were detected, although the experiment was limited by severe toxicity of the treatment. p53 immunostaining was done and there was no difference in transgenic and non-transgenic mice. These results suggest that O6-methylguanine formation might not be a significant **mutational** factor in these mouse urinary bladder carcinogenesis models.

Tags: Female; Human; Male; Support, Non-U.S. Gov't

Descriptors: Bladder Neoplasms--genetics--GE; *Genes, p53; *Guanine --analogs and derivatives--AA; * **Mutation** ; *O(6)-Methylguanine-DNA Methyltransferase--metabolism--ME; Adenosine Deaminase--genetics--GE; Adenosine Deaminase--metabolism--ME; Animals; Bladder--drug effects--DE; Bladder--pathology--PA; Bladder Neoplasms--chemically induced--CI; Bladder Neoplasms--epidemiology--EP; Bladder Neoplasms--pathology--PA; Butylhydroxybutylnitrosamine; Carcinogens; Carcinoma, Squamous Cell --chemically induced--CI; Carcinoma, Squamous Cell--epidemiology--EP; Carcinoma, Squamous Cell--genetics--GE; Carcinoma, Squamous Cell --pathology--PA; Carcinoma, Transitional Cell--chemically induced--CI; Carcinoma, Transitional Cell--epidemiology--EP; Carcinoma, Transitional Cell--genetics--GE; Carcinoma, Transitional Cell--pathology--PA; Epithelium--drug effects--DE; Epithelium--pathology--PA; Guanine --metabolism--ME; Incidence; Mice; Mice, Inbred C3H; Mice, Transgenic; Neoplasm Invasiveness; O(6)-Methylguanine-DNA Methyltransferase--genetics --GE; Polymerase Chain Reaction; Polymorphism, Single-Stranded Conformational

CAS Registry No.: 0 (Carcinogens); 20535-83-5 (O-(6)-methylguanine); 3817-11-6 (Butylhydroxybutylnitrosamine); 73-40-5 (Guanine)

Enzyme No.: EC 2.1.1.63 (O(6)-Methylguanine-DNA Methyltransferase); EC 3.5.4.4 (Adenosine Deaminase)

Record Date Created: 19981116

Record Date Completed: 19981116

8/9/2

DIALOG(R)File 155:MEDLINE(R)

13953474 PMID: 9654068

Methanol:coenzyme M methyltransferase from Methanosarcina barkeri--identification of the active-site histidine in the corrinoid-harboring subunit MtaC by site-directed mutagenesis .

Sauer K; Thauer R K

Max-Planck-Institut fur terrestrische Mikrobiologie and Laboratorium fur Mikrobiologie des Fachbereichs Biologie der Philipps-Universitat, Marburg, Germany.

European journal of biochemistry / FEBS (GERMANY) May 1 1998, 253 (3) p698-705, ISSN 0014-2956 Journal Code: 0107600

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The enzyme system catalyzing the formation of methyl-coenzyme M from methanol and coenzyme M in Methanosarcina barkeri is composed of the three different polypeptides MtaA, MtaB and MtaC of which MtaC harbors a corrinoid prosthetic group. The **heterologous** expression of mtaA and mtaB

in Escherichia coli has been described previously. We report here on the overproduction of the apoprotein of MtaC in E. coli, on its reconstitution to the active holoprotein with either cob(II)alamin or methyl-cob(III)alamin, and on the properties of the reconstituted corrinoid protein. Reconstituted MtaC was found to contain 1 mol bound cobamide/mol. EPR spectroscopic evidence is presented for a His residue as an axial ligand to Co2+ of the bound corrinoid. This active-site His was identified by site-directed **mutagenesis** as His136 in the MtaC sequence that contains four His residues. The reconstituted MtaC, in the cob(I)amide oxidation state, was methylated with methanol in the presence of MtaB and demethylated with coenzyme M in the presence of MtaA. In the presence of both MtaB and MtaA, methyl-coenzyme M was formed from methanol and coenzyme M at specific rates comparable to those determined for the enzyme system purified from M. barkeri. M. barkeri contains an isoenzyme of MtaA designated MtbA. The isoenzyme reacted with MtaC with only 2.5% of the activity of MtaA.

Tags: Support, Non-U.S. Gov't

Descriptors: Adenosinetriphosphatase--chemistry--CH; *Adenosinetriphosphatase--metabolism--ME; * **Carrier** Proteins--chemistry--CH; * **Carrier** Proteins--metabolism--ME; *Histidine; *Methanosarcina barkeri--enzymology--EN; *Methyltransferases--chemistry--CH; *Methyltransferases--metabolism--ME; Adenosinetriphosphatase--isolation and purification--IP; Apoproteins--chemistry--CH; Apoproteins--metabolism--ME; Base Sequence; Binding Sites; **Carrier** Proteins--isolation and purification--IP; Cloning, Molecular; DNA Primers; Electron Spin Resonance Spectroscopy; Escherichia coli; Isoenzymes--chemistry--CH; Isoenzymes--isolation and purification--IP; Isoenzymes--metabolism--ME; Kinetics; Macromolecular Systems; Methyltransferases--isolation and purification--IP; **Mutagenesis**, Site-Directed; Protein Conformation; Recombinant Proteins--chemistry--CH; Recombinant Proteins--isolation and purification--IP; Recombinant Proteins--metabolism--ME

CAS Registry No.: 0 (Apoproteins); 0 (Carrier Proteins); 0 (DNA Primers); 0 (Isoenzymes); 0 (Macromolecular Systems); 0 (Mta72 protein); 0 (Recombinant Proteins); 71-00-1 (Histidine)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (methanol-2-mercaptoethanesulfonic acid methyltransferase); EC 3.6.1.3 (Adenosinetriphosphatase)

Record Date Created: 19980729

Record Date Completed: 19980729

8/9/3

DIALOG(R)File 155:MEDLINE(R)

13928083 PMID: 9628345

Functional analysis of conserved motifs in type III restriction-modification enzymes.

Saha S; Ahmad I; Reddy Y V; Krishnamurthy V; Rao D N

Department of Biochemistry, Indian Institute of Science, Bangalore.

Biological chemistry (GERMANY) Apr-May 1998, 379 (4-5) p511-7, ISSN 1431-6730 Journal Code: 9700112

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

EcoP1I and EcoP15I are members of type III restriction-modification enzymes. EcoPI and EcoP15I DNA methyltransferases transfer a methyl group from S-adenosyl-L-methionine (AdoMet) to the N6 position of the second adenine residues in their recognition sequences, 5'-AGACC-3' and 5'-CAGCAG-3' respectively. We have **altered** various residues in two highly conserved sequences, FxGxG (motif I) and DPPY (motif IV) in these proteins by site-directed **mutagenesis**. Using a mixture of in vivo and in vitro assays, our results on the **mutational** analysis of these methyltransferases demonstrate the universal role of motif I in AdoMet binding and a role for motif IV in catalysis. All six cysteine residues in

Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--isolation and purification--IP; Temperature
CAS Registry No.: 0 (DNA Transposable Elements)
Enzyme No.: EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19980713
Record Date Completed: 19980713

8/9/5

DIALOG(R)File 155:MEDLINE(R)

13835291 PMID: 9535090
Control of expression of LlaI restriction in Lactococcus lactis.
O'Sullivan D J; Klaenhammer T R
Department of Food Science, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh 27695-7624, USA.
Molecular microbiology (ENGLAND) Mar 1998, 27 (5) p1009-20, ISSN 0950-382X Journal Code: 8712028
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The **plasmid** encoded LlaI R/M system from Lactococcus lactis ssp. lactis consists of a bidomain methylase, with close evolutionary ties to type IIS methylases, and a trisubunit restriction complex. Both the methylase and restriction subunits are encoded on a polycistronic 6.9 kb operon. In this study, the 5' end of the llal 6.9 kb transcript was determined by primer extension analysis to be 254 bp upstream from the first R/M gene on the operon, llalM. Deletion of this promoter region abolished LlaI restriction in L. lactis. Analysis of the intervening sequence revealed a 72-amino-acid open reading frame, designated llalC, with a conserved ribosome binding site and helix-turn-helix domain. Overexpression of llalC in Escherichia coli with a T7 expression **vector** produced the predicted protein of 8.2 kDa. **Mutation** and in trans complementation analyses indicated that C-LlaI positively enhanced LlaI restriction activity in vivo. Northern analysis and transcriptional fusions of the llal promoter to a lacZ reporter gene indicated that C x LlaI did not enhance transcription of the llal operon. Databank searches with the deduced protein sequence for llalC revealed significant homologies to the E. coli Rop regulatory and mRNA stabilizer protein. Investigation of the effect of C x LlaI on enhancement of LlaI restriction in L. lactis revealed that growth at elevated temperatures (40 degrees C) completely abolished any enhancement of restriction activity. These data provide molecular evidence for a mechanism on how the expression of a restriction system in a prokaryote can be drastically **reduced** during elevated growth temperatures, by a small regulatory protein.
Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.
Descriptors: **Carrier** Proteins--genetics--GE; *Gene Expression Regulation, Bacterial; *Lactococcus lactis--genetics--GE; *Operon; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Amino Acid Sequence; **Carrier** Proteins--chemistry--CH; Cloning, Molecular; Gene Fusion; Genetic Complementation Test; Lac Operon; Lactococcus lactis --enzymology--EN; Molecular Sequence Data; **Mutation** ; Open Reading Frames; **Plasmids** ; Polymerase Chain Reaction; Promoter Regions (Genetics); RNA --isolation and purification--IP; RNA Processing, Post-Transcriptional; Transcription, Genetic; Translation, Genetic
CAS Registry No.: 0 (Carrier Proteins); 0 (LlaIC protein); 0 (Plasmids); 63231-63-0 (RNA)
Enzyme No.: EC 2.1.1.- (DNA modification methylase LlaI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19980609
Record Date Completed: 19980609

8/9/6

DIALOG(R)File 155:MEDLINE(R)

13754804 PMID: 9449951

Molecular analysis of the macrolide-lincosamide resistance gene region of a novel plasmid **from Staphylococcus hyicus.**

Schwarz S; Lange C; Werckenthin C

Institut fur Kleintierforschung Celle/Merbitz, Bundesforschungsanstalt fur Landwirtschaft Braunschweig-Volkenrode (FAL), Germany.

Journal of medical microbiology (ENGLAND) Jan 1998, 47 (1) p63-70, ISSN 0022-2615 Journal Code: 0224131

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Resistance to macrolides and lincosamides in Staphylococcus hyicus has been shown to be encoded by a 4.0-kb **plasmid** designated pSES21. It differed distinctly in its restriction map from all other staphylococcal macrolide resistance **plasmids** reported so far. Southern blot hybridisation with gene probes specific for staphylococcal erm genes demonstrated that the macrolide resistance gene belonged to hybridisation class C. Analysis of the ermC gene revealed that the deduced amino-acid sequence of the pSES21-encoded ErmC methylase exhibited c. 93% identity with the ErmC methylase encoded by **plasmid** pE194. The ermC gene of pSES21 was expressed constitutively and sequence analysis of the regulatory region showed multiple base-pair **insertions** and substitutions in the translational **attenuator** . As a consequence of these **mutations** , the reading frame of the small regulatory peptide was destroyed and a novel pair of inverted repeated sequences was generated. Previous studies identified sequence deletions and sequence duplications in the ermC regulatory region as the basis for constitutive ermC gene expression. The multiple point **mutations** shown in the pSES21-encoded ermC translational **attenuator** represent a novel kind of structural **alteration** in this regulatory region and may explain constitutive ermC gene expression by pairing of the newly generated inverted repeated segments in the presence of a functionally deleted reading frame for the small regulatory peptide.

Tags: Support, Non-U.S. Gov't

Descriptors: Anti-Bacterial Agents--pharmacology--PD; * **Plasmids** --genetics--GE; *Staphylococcus--drug effects--DE; Amino Acid Sequence; Animals; Base Sequence; Blotting, Southern; DNA, Bacterial--chemistry--CH; DNA, Bacterial--genetics--GE; Drug Resistance, Microbial--genetics--GE; Gene Expression Regulation, Bacterial; Macrolides--pharmacology--PD; Methyltransferases--chemistry--CH; Methyltransferases--genetics--GE; Molecular Sequence Data; **Plasmids** --chemistry--CH; Point **Mutation** ; Reading Frames; Repetitive Sequences, Nucleic Acid; Restriction Mapping; Staphylococcus--genetics--GE; Swine; Transcription, Genetic; Translation, Genetic

CAS Registry No.: 0 (Anti-Bacterial Agents); 0 (DNA, Bacterial); 0 (Macrolides); 0 (Plasmids); 80738-43-8 (lincosamide)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.66 (rRNA (**adenosine** -O-2'-) **methyltransferase**)

Record Date Created: 19980217

Record Date Completed: 19980217

8/9/7

DIALOG(R)File 155:MEDLINE(R)

13670978 PMID: 9379896

Overinitiation of chromosome replication in the Escherichia coli dnaAcos mutant **depends on activation of oriC function by the dam gene product.**

Katayama T; Akimitsu N; Mizushima T; Miki T; Sekimizu K

Department of Microbiology, Kyushu University Faculty of Pharmaceutical Sciences, Fukuoka, Japan.

Molecular microbiology (ENGLAND) Aug 1997, 25 (4) p661-70, ISSN

0950-382X Journal Code: 8712028
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The activity of DnaA protein, the initiator of chromosome replication in Escherichia coli, is regulated by adenine nucleotide binding; the ATP-bound form, not the ADP-bound form, is active. DnaAcos is a **mutant** protein that is insensitive to **negative** regulation by ADP. Initiation of chromosome replication occurs excessively in the dnaAcos **mutant** at 30 degrees C, a restrictive temperature for growth. To determine the control factors that act independently of adenine nucleotide binding of DnaA, we analysed suppressors from the dnaAcos **mutant** isolated by Tn5 **insertion mutagenesis** . Three of the suppressors **carried** Tn5 in the aroK or aroB gene, the first two cistrons in the dam operon. Complementation tests revealed that the dam gene is responsible for the suppression. Over-replication of the chromosome was inhibited in the dnaAcos aroK::Tn5 double **mutant** , and initiation of chromosome replication in the dnaA+ aroK::Tn5 **mutant** was partially inhibited. The aroK(or B)::Tn5 cells contained DnaA molecules at a level similar to that in the parental aroBK+ strain. Moreover, dnaAcos suppression depended on the function of the seqA gene. Thus, Dam activity positively regulates initiation of chromosome replication in vivo. SeqA function seems to be distinguished from the control of DnaA protein by adenine nucleotide binding.

Tags: Support, Non-U.S. Gov't

Descriptors: Bacterial Proteins--genetics--GE; *DNA Replication--genetics --GE; *DNA-Binding Proteins--genetics--GE; *Escherichia coli--genetics--GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME ; *Transcription Factors; Adenosine Diphosphate--pharmacology--PD; Bacterial Proteins--metabolism--ME; Blotting, Western; Chloramphenicol --pharmacology--PD; Chromosomes, Bacterial; Cloning, Molecular; DNA Transposable Elements--genetics--GE; Escherichia coli--metabolism--ME; Flow Cytometry; Genetic Complementation Test; **Mutagenesis , Insertional** --genetics--GE; Phenotype; **Plasmids** --genetics--GE; Replication Origin --genetics--GE; Sequence Analysis, DNA; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Suppression, Genetic

CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA Transposable Elements); 0 (DNA-Binding Proteins); 0 (Plasmids); 0 (SeqA protein); 0 (Transcription Factors); 0 (dnaA protein); 56-75-7 (Chloramphenicol) ; 58-64-0 (Adenosine Diphosphate)

Enzyme No.: EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19971112
Record Date Completed: 19971112

8/9/8
DIALOG(R)File 155:MEDLINE(R)

13594948 PMID: 9278491

Substrate DNA and cofactor regulate the activities of a multi-functional restriction-modification enzyme, BcgI.

Kong H; Smith C L
New England Biolabs, 32 Tozer Road, Beverly, MA 01915, USA. kong@neb.com

Nucleic acids research (ENGLAND) Sep 15 1997, 25 (18) p3687-92, ISSN 0305-1048 Journal Code: 0411011

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The BcgI restriction-modification system consists of two subunits, A and B. It is a bifunctional protein complex which can cleave or methylate DNA. The regulation of these competing activities is determined by the DNA

substrates and cofactors. BcgI is an active endonuclease and a poor methyltransferase on unmodified DNA substrates. In contrast, BcgI is an active methyltransferase and an inactive endonuclease on hemimethylated DNA substrates. The cleavage and methylation reactions share cofactors. While BcgI requires Mg2+and S -adenosyl methionine (AdoMet) for DNA cleavage, its methylation reaction requires only AdoMet and yet is significantly stimulated by Mg2+. Site-directed **mutagenesis** was **carried** out to investigate the relationship between AdoMet binding and BcgI DNA cleavage/methylation activities. Most substitutions of conserved residues forming the AdoMet binding pocket in the A subunit abolished both methylation and cleavage activities, indicating that AdoMet binding is an early common step required for both cleavage and methylation. However, one **mutation** (Y439A) abolished only the methylation activity, not the DNA cleavage activity. This **mutant** protein was purified and its methylation, cleavage and AdoMet binding activities were tested in vitro . BcgI-Y439A had no detectable methylation activity, but it retained 40% of the AdoMet binding and DNA cleavage activities.

Descriptors: DNA, Bacterial--metabolism--ME; *Deoxyribonucleases, Type II Site-Specific--metabolism--ME; *Escherichia coli--enzymology--EN; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME; Base Sequence; Enzyme Activation; Escherichia coli--genetics--GE; Molecular Sequence Data; Substrate Specificity

CAS Registry No.: 0 (DNA, Bacterial)

Enzyme No.: EC 2.1.1.- (BcgI methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (endodeoxyribonuclease BcgI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)

Record Date Created: 19971017

Record Date Completed: 19971017

8/9/9

DIALOG(R)File 155:MEDLINE(R)

13528984 PMID: 9215582

Molecular analysis of naturally occuring ermC-encoding plasmids **in staphylococci isolated from animals with and without previous contact with macrolide/lincosamide antibiotics.**

Lodder G; Werckenthin C; Schwarz S; Dyke K

Institut fur Kleintierforschung der Bundesforschunganstalt fur Landwirtschaft (FAL), Celle, Germany.

FEMS immunology and medical microbiology (NETHERLANDS) May 1997, 18 (1) p7-15, ISSN 0928-8244 Journal Code: 9315554

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A total of 16 epidemiologically unrelated macrolide-resistant staphylococcal isolates of various animal origins were investigated for the molecular basis of macrolide resistance with respect to previous contact of their host animals with macrolides and lincosamides. All isolates **carried** ermC-encoding **plasmids** of 2.3-4.0 kbp. The eight **plasmids** of staphylococci from animals which had not received macrolides or lincosamides showed inducible ermC gene expression and did not exhibit **alterations** in the ermC regulatory region. The remaining eight **plasmids** expressed the ermC gene constitutively. Six of these **plasmids** were from staphylococci from animals which had received tylosin or spiramycin as feed additives or lincomycin for therapeutic purposes. All constitutively expressed ermC genes revealed either sequence deletions or sequence duplications in their ermC regulatory region, as detected by a PCR assay and by sequence analysis. These sequence deletions and duplications found in naturally occurring **plasmids** corresponded closely to the **mutations** seen in the ermC-encoding **plasmids** after growth of an inducibly resistant strain in the presence of non-inducing macrolides or lincosamides under in vitro conditions.

Tags: Support, Non-U.S. Gov't
Descriptors: Anti-Bacterial Agents--pharmacology--PD; *Drug Resistance, Microbial--genetics--GE; *Methyltransferases--genetics--GE; * **Plasmids** --genetics--GE; *Staphylococcal Infections--veterinary--VE; *Staphylococcus --genetics--GE; Amino Acid Sequence; Animals; Anti-Bacterial Agents --therapeutic use--TU; Base Sequence; Gene Expression Regulation, Bacterial ; Macrolides--pharmacology--PD; Macrolides--therapeutic use--TU; Molecular Sequence Data; Polymerase Chain Reaction; Regulatory Sequences, Nucleic Acid; Restriction Mapping; Staphylococcal Infections--drug therapy--DT; Staphylococcal Infections--microbiology--MI; Staphylococcus--drug effects --DE; Staphylococcus--isolation and purification--IP
Molecular Sequence Databank No.: GENBANK/X82668; GENBANK/Y09001; GENBANK/Y09002
CAS Registry No.: 0 (Anti-Bacterial Agents); 0 (Macrolides); 0 (Plasmids); 80738-43-8 (lincosamide)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.66 (rRNA (**adenosine** -O-2'-) **methyltransferase**)
Record Date Created: 19971023
Record Date Completed: 19971023

8/9/10
DIALOG(R)File 155:MEDLINE(R)

13391839 PMID: 9065822
The relationship of a novel drug-resistant phenotype in C3H10T1/2 cells selected with alkylating agents to neoplastic transformation **and ATP metabolism.**
Wroblewski-Giorgio D; von Hofe E
Hybridon, Inc., Worcester, MA 01605, USA.
Cancer letters (IRELAND) Feb 26 1997, 113 (1-2) p195-203, ISSN 0304-3835 Journal Code: 7600053
Contract/Grant No.: CA 51103; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
We investigated the resistance to alkylating agents in parental, drug-selected and neoplastically **transformed** C3H10T1/2 (10T1/2) murine fibroblasts. Similar levels of resistance to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) were observed in cells selected for resistance to MNNG or 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) as well as in cells **transformed** by a single treatment with MNNG. Surprisingly, neither the levels of O6-alkylguanine-DNA alkyltransferase (AT) nor glutathione-S-transferase (GST) were **altered** in drug-resistant cells. In contrast, changes in ATP metabolism were observed in both **transformed** and MNNG-selected cells after treatment with MNNG. Specifically, 3 h after treatment with 5 microg/ml MNNG, ATP levels decreased by 85% and 74% in MNNG-selected and **transformed** cells, respectively, compared to only a 28% decrease in parental cells. Therefore, rather than contributing to cell sensitivity to alkylating agents, the ability to rapidly utilize ATP and tolerate resulting decreases in ATP levels may in some cases play a role in protection from the cytotoxic effects of alkylating agents.
Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Descriptors: *Adenosine Triphosphate--metabolism--ME; *Antineoplastic Agents, Alkylating--pharmacology--PD; *Carmustine--pharmacology--PD; *Methylnitronitrosoguanidine--pharmacology--PD; Animals; Cell Survival --drug effects--DE; Cells, Cultured; Drug Resistance; Fibroblasts--drug effects--DE; Fibroblasts--enzymology--EN; Fibroblasts--metabolism--ME; Glutathione--metabolism--ME; Glutathione Transferase--metabolism--ME; Methyltransferases--metabolism--ME; Mice; O(6)-Methylguanine-DNA Methyltransferase
CAS Registry No.: 0 (Antineoplastic Agents, Alkylating); 154-93-8 (Carmustine); 56-65-5 (Adenosine Triphosphate); 70-18-8 (Glutathione); 70-25-7 (Methylnitronitrosoguanidine)

mismatch repair. However, differences between S. typhimurium and E. coli dam **mutants** are also found: (1) S. typhimurium dam **mutants** do not show increased UV sensitivity, suggesting that methyl-directed mismatch repair does not participate in the repair of UV-induced DNA damage in Salmonella. (2) S. typhimurium dam recJ **mutants** are viable, suggesting that the Salmonella RecJ function does not participate in the repair of DNA strand breaks formed in the absence of Dam methylation. We also describe a genetic screen for detecting novel genes regulated by Dam methylation and a locus repressed by Dam methylation in the S. typhimurium virulence (or "cryptic") **plasmid** .

Tags: Support, Non-U.S. Gov't

Descriptors: Salmonella typhimurium--enzymology--EN; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Chromosome Mapping; Cloning, Molecular; DNA Methylation; DNA Transposable Elements; Gene Deletion; Genetic Complementation Test; **Mutagenesis , Insertional** ; Salmonella typhimurium--genetics--GE; Salmonella typhimurium--radiation effects--RE; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific) --metabolism--ME; Ultraviolet Rays

CAS Registry No.: 0 (DNA Transposable Elements)

Enzyme No.: EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19970128

Record Date Completed: 19970128

8/9/14

DIALOG(R)File 155:MEDLINE(R)

13144278 PMID: 8813050

Both an altered **DNA structure and cellular proteins are involved in protecting a triplex forming an oligopurine-rich sequence from Dam methylation in E. coli.**

Klysik J

Institute of Biosciences and Technology, Texas A&M University, Texas Medical Center, Houston 77030, USA.

Biochemical genetics (UNITED STATES) Jun 1996, 34 (5-6) p165-78, ISSN 0006-2928 Journal Code: 0126611

Contract/Grant No.: AI28071; AI; NIAID; NS32583; NS; NINDS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

When the 4-bp Dam recognition sequence was placed between two d(GA)7 tracts, it became severely undermethylated in JM101 Escherichia coli cells compared to other Dam sequences in the same **plasmid** DNA. This site specific undermethylation was also detected on supercoiled molecules in vitro. **Mutational** analysis indicated that undermethylation is related to the capacity of the oligopurine tract to adopt the H-DNA conformation. In addition, chemical probing of the cells was consistent with a cellular protein bound to the DNA. Therefore it is likely that the combination of **altered** DNA conformation and a cellular protein leads to Dam-site protection. We also found that the site-specific undermethylation is detectable in certain E. coli strains only.

Tags: Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.

Descriptors: Bacterial Proteins--metabolism--ME; *DNA, Bacterial --metabolism--ME; *Escherichia coli--metabolism--ME; *Purines; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME ; Base Sequence; Binding Sites; DNA Footprinting; DNA Methylation; DNA, Bacterial--chemistry--CH; DNA, Single-Stranded--metabolism--ME; DNA, Superhelical--metabolism--ME; Escherichia coli--genetics--GE; Molecular Sequence Data; Nucleic Acid Conformation; Oligodeoxyribonucleotides; Structure-Activity Relationship

CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA, Bacterial); 0 (DNA, Single-Stranded); 0 (DNA, Superhelical); 0 (Oligodeoxyribonucleotides); 0 (Purines); 120-73-0 (purine)

Enzyme No.: EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19961107
Record Date Completed: 19961107

8/9/15
DIALOG(R)File 155:MEDLINE(R)

13119550 PMID: 8787908
Tandem duplication in ermC translational attenuator **of the macrolide-lincosamide-streptogramin B resistance** plasmid **pSES6 from Staphylococcus equorum.**
Lodder G; Schwarz S; Gregory P; Dyke K
Institut fur Kleintierforschung Celle/Merbitz, Bundesforschungsanstalt fur Landwirtschaft Braunschweig-Volkenrode, Celle, Germany.
Antimicrobial agents and chemotherapy (UNITED STATES) Jan 1996, 40 (1) p215-7, ISSN 0066-4804 Journal Code: 0315061
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
A tandem duplication of 23 bp in the ermC gene translational **attenuator** of **plasmid** pSES6 from Staphylococcus equorum which mediated constitutive resistance to macrolide-lincosamide-streptogramin B antibiotics was identified. This duplication included the ribosome binding site for the ermC gene as well as the first 5 bp of the ermC coding sequence. It was postulated that this sequence duplication affects the possible RNA conformations so that the ribosome binding site for ErmC synthesis is readily accessible to the ribosomes and thus constitutive expression of the ermC gene occurs.
Tags: Support, Non-U.S. Gov't
Descriptors: *Anti-Bacterial Agents--pharmacology--PD; *Antibiotics, Peptide--pharmacology--PD; *Methyltransferases--genetics--GE; *Repetitive Sequences, Nucleic Acid--drug effects--DE; *Staphylococcus--genetics--GE; *Translation, Genetic--drug effects--DE; *Virginiamycin--pharmacology--PD; Amino Acid Sequence; Base Sequence; Cloning, Molecular; Drug Resistance, Microbial--genetics--GE; Genes, Bacterial--drug effects--DE; Macrolides --pharmacology--PD; Molecular Sequence Data; **Plasmids** --drug effects--DE; **Plasmids** --genetics--GE; Staphylococcus--drug effects--DE
Molecular Sequence Databank No.: GENBANK/X82664; GENBANK/X82665; GENBANK/X82666; GENBANK/X82667; GENBANK/X82668
CAS Registry No.: 0 (Anti-Bacterial Agents); 0 (Antibiotics, Peptide); 0 (Macrolides); 0 (Plasmids); 11006-76-1 (Virginiamycin); 80738-43-8 (lincosamide)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.66 (rRNA (**adenosine** -O-2'-) **methyltransferase**)
Record Date Created: 19970123
Record Date Completed: 19970123

8/9/16
DIALOG(R)File 155:MEDLINE(R)

13002899 PMID: 8675023
Plasmids **with erythromycin resistance and catechol 2,3-dioxygenase- or beta-galactosidase-encoding gene cassettes for use in Neisseria spp.**
Zhou D; Apicella M A
Department of Microbiology, The University of Iowa, Iowa City 52242, USA.
Gene (NETHERLANDS) May 24 1996, 171 (1) p133-4, ISSN 0378-1119 Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
Subfile: INDEX MEDICUS

Four new **plasmids** containing the ermC' (encoding a methyltransferase which confers resistance to erythromycin), xylE-ermC' (xylE, encoding catechol 2,3-dioxygenase) and lacZ-ermC' cassettes have been constructed. The 10-bp gonococcal uptake sequence has been placed downstream from ermC' to facilitate the delivery of these cassettes into pathogenic Neisseria spp. Several restriction sites have been placed to flank the cassettes to allow their excision and directional cloning. These **plasmids** will provide valuable tools for constructing **insertional mutants** and transcriptional fusions in Neisseria spp. or other bacteria.

Descriptors: Genetic **Vectors** --genetics--GE; *Lac Operon--genetics--GE; *Methyltransferases--genetics--GE; *Neisseria--genetics--GE; *Oxygenases --genetics--GE; Anti-Bacterial Agents; Base Sequence; Cloning, Molecular --methods--MT; DNA, Recombinant; Drug Resistance, Microbial; Erythromycin; Molecular Sequence Data; **Transformation** , Bacterial

CAS Registry No.: 0 (Anti-Bacterial Agents); 0 (DNA, Recombinant); 0 (Genetic Vectors); 114-07-8 (Erythromycin)

Enzyme No.: EC 1.13. (Oxygenases); EC 1.13.11.2 (catechol-2,3-dioxygenase); EC 2.1.1. (Methyltransferases); EC 2.1.1.66 (rRNA (**adenosine** -O-2'-) **methyltransferase**)

Record Date Created: 19960812
Record Date Completed: 19960812

8/9/17
DIALOG(R)File 155:MEDLINE(R)

12885885 PMID: 8552587

Influence of alkyltransferase activity and chromosomal locus on mutational hotspots in Chinese hamster ovary cells.

Belouchi A; Ouimet M; Dion P; Gaudreault N; Bradley W E

Institut du cancer de Montreal, Centre de Recherche Louis-Charles Simard, Montreal, Canada.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Jan 9 1996, 93 (1) p121-5, ISSN 0027-8424
Journal Code: 7505876

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

High-density **mutational** spectra have been established for exon 3 of the gene encoding adenine phosphoribosyltransferase (APRT) of the Chinese hamster ovary (CHO) cell line derivative D422 and closely related and/or modified lines by using the mutagen ethyl methanesulfonate (EMS). The total number of selectable sites (GC-->AT transitions yielding a selectable APRT- phenotype) was estimated at 31 based on our own accumulated data base of 136 sequenced exon 3 **mutations** and on literature reports. D422 and two other APRT hemizygous lines each yielded very similar spectra and showed two populations of mutable sites: (i) 24 "baseline" sites that followed the Poisson distribution and therefore were equally susceptible to **mutation** and (ii) two hotspots, one comprising a cluster at nucleotides 1293-1309 and the other at nucleotide 1365. Collectively, the latter sites were about 10-fold more frequently mutated than the others. CHO cells are mer- as they lack the repair enzyme O6-methylguanidine methyltransferase (EC 2.1.1.63). In modified repair-proficient CHO cells, the distribution of **mutations** among all of the 31 sites was random, with only 3 of the 19 GC-->AT transitions in the above hotspots. To determine whether the distribution was locus-dependent, two independent lines **carrying** single copies of transfected APRT genes were generated from a derivative of D422 **carrying** a deletion in the endogenous APRT gene. Nucleotides 1293-1309 were again no longer preferentially mutated, but the site at nucleotide 1365 was still a hotspot. We conclude that **mutational** spectra in mer- cells are at least in part locus dependent and that some sequences are particularly susceptible to EMS **mutagenesis** and perhaps also to methyltransferase

repair.
Tags: Support, Non-U.S. Gov't
Descriptors: *Methyltransferases--metabolism--ME; Adenine Phosphoribosyltransferase--genetics--GE; Animals; Base Composition; Base Sequence; CHO Cells; DNA Repair; Ethyl Methanesulfonate--chemistry--CH; Exons; Hamsters; Molecular Sequence Data; Mutagens--chemistry--CH; O(6)-Methylguanine-DNA Methyltransferase; Point **Mutation** ; Polymorphism, Single-Stranded Conformational; Structure-Activity Relationship
CAS Registry No.: 0 (Mutagens); 62-50-0 (Ethyl Methanesulfonate)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.63 (O(6)-Methylguanine-DNA Methyltransferase); EC 2.4.2.7 (Adenine Phosphoribosyltransferase)
Record Date Created: 19960222
Record Date Completed: 19960222

8/9/18
DIALOG(R)File 155:MEDLINE(R)

12872554 PMID: 7498762
The diversity of alleles at the hsd locus in natural populations of Escherichia coli.
Barcus V A; Titheradge A J; Murray N E
Institute of Cell and Molecular Biology, University of Edinburgh, United Kingdom.
Genetics (UNITED STATES) Aug 1995, 140 (4) p1187-97, ISSN 0016-6731 Journal Code: 0374636
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
In enteric bacteria three discrete families of type I restriction and modification systems (IA, IB and ID) are encoded by alleles of the serB-linked hsd locus. Probes specific for each of the three families were used to monitor the distribution of related systems in 37 of the 72 wild-type Escherichia coli strains comprising the ECOR collection. All 25 members of group A in this collection were screened; 12 were probe-positive, nine have hsd genes in the IA family, two in the IB and one in the ID. Twelve strains, representing all groups other than A, were screened; five were probe-positive, one has hsd genes in the IA family, one in the IB and three in the ID. The type ID genes are the first representatives of this family in E. coli, the probe- **negative** strains could have **alternative** families of hsd genes. The type IA and IB systems added at least five new specificities to the five already identified in natural isolates of E. coli. The distribution of alleles is inconsistent with the dendrogram of the bacterial strains derived from other criteria. This discrepancy and the dissimilar coding sequences of allelic hsd genes both imply lateral transfer of hsd genes.
Tags: Comparative Study; Support, Non-U.S. Gov't
Descriptors: Alleles; *Deoxyribonucleases, Type I Site-Specific--genetics--GE; *Escherichia coli--genetics--GE; *Genes, Structural, Bacterial; *Multigene Family; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Base Sequence; Cloning, Molecular; DNA, Bacterial--genetics--GE; Escherichia coli--classification--CL; Escherichia coli--enzymology--EN; Escherichia coli--isolation and purification--IP; Evolution, Molecular; Molecular Sequence Data; Nucleic Acid Hybridization; Selection (Genetics); Species Specificity; Substrate Specificity; **Transformation** , Bacterial; Variation (Genetics)
CAS Registry No.: 0 (DNA, Bacterial)
Enzyme No.: EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.3 (Deoxyribonucleases, Type I Site-Specific)
Record Date Created: 19960117
Record Date Completed: 19960117

8/9/19
DIALOG(R)File 155:MEDLINE(R)

12865194 PMID: 8655647

Analysis of the temporal program of replication initiation in yeast chromosomes.

Friedman K L; Raghuraman M K; Fangman W L; Brewer B J
Department of Genetics, University of Washington, Seattle 98195-7360, USA.

Journal of cell science. Supplement (ENGLAND) 1995, 19 p51-8, ISSN 0269-3518 Journal Code: 8502898
Contract/Grant No.: 18926; PHS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The multiple origins of eukaryotic chromosomes vary in the time of their initiation during S phase.In the chromosomes of Saccharomyces cerevisiae the presence of a functional telomere causes nearby origins to delay initiation until the second half of S phase. The key feature of telomeres that causes the replication delay is the telomeric sequence (C(1-3)A/G(1-3)T) itself and not the proximity of the origin to a DNA end. A second group of late replicating origins has been found at an internal position on chromosome XIV. Four origins, spanning approximately 140 kb, initiate replication in the second half of S phase. At least two of these internal origins maintain their late replication time on circular **plasmids**. Each of these origins can be separated into two functional elements: those sequences that provide origin function and those that impose late activation. Because the assay for determining replication time is costly and laborious, it has not been possible to analyze in detail these 'late' elements. We report here the development of two new assays for determining replication time. The first exploits the expression of the Escherichia coli dam methylase in yeast and the characteristic period of hemimethylation that transiently follows the passage of a replication fork. The second uses quantitative hybridization to detect two-fold differences in the amount of specific restriction fragments as a function of progress through S phase. The novel aspect of this assay is the creation in vivo of a non-replicating DNA sequence by site-specific pop-out recombination. This non-replicating fragment acts as an internal control for copy number within and between samples. Both of these techniques are rapid and much less costly than the more conventional density transfer experiments that require CsCl gradients to detect replicated DNA. With these techniques it should be possible to identify the sequences responsible for late initiation, to search for other late replicating regions in the genome, and to begin to analyze the effect that **altering** the temporal program has on chromosome function.

Tags: Support, U.S. Gov't, P.H.S.
Descriptors: *Chromosomes, Fungal--physiology--PH; *DNA Replication --genetics--GE; *DNA, Fungal--genetics--GE; *Saccharomyces cerevisiae --genetics--GE; Blotting, Southern; DNA Nucleotidyltransferases--metabolism --ME; Escherichia coli--enzymology--EN; Fungal Proteins--metabolism--ME; Replication Origin--genetics--GE; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--biosynthesis--BI; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME
CAS Registry No.: 0 (DNA, Fungal); 0 (Fungal Proteins)
Enzyme No.: EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 2.7.7.- (DNA Nucleotidyltransferases); EC 2.7.7.- (R recombinase)
Record Date Created: 19960731
Record Date Completed: 19960731

8/9/20
DIALOG(R)File 155:MEDLINE(R)

12814699 PMID: 7590303

The thiostrepton-resistance-encoding gene in Streptomyces laurentii is located within a cluster of ribosomal protein operons.

Smith T M; Jiang Y F; Shipley P; Floss H G

Department of Medicinal Chemistry BG-20, University of Washington, Seattle 98195, USA.

Gene (NETHERLANDS) Oct 16 1995, 164 (1) p137-42, ISSN 0378-1119 Journal Code: 7706761

Contract/Grant No.: AI20264; AI; NIAID; GM 07750; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A common approach to identify and clone biosynthetic gene from an antibiotic-producing streptomycete is to clone the resistance gene for the antibiotic of interest and then use that gene to clone DNA that is linked to it. As a first step toward cloning the genes responsible for the biosynthesis of thiostrepton (Th) in Streptomyces laurentii (Sl), the Th resistance-encoding gene (tsnR) was cloned as a 1.5-kb BamHI-PvuII fragment in Escherichia coli (Ec), and shown to confer Th resistance when introduced into S. lividans TK24. The tsnR-containing DNA fragment was used as a probe to isolate clones from cosmid libraries of DNA in the Ec cosmid **vector** SuperCos, and pOJ446 (an Ec/streptomycete) cosmid **vector** . Sequence and genetic analysis of the DNA flanking the tsnR indicates that the Sl tsnR is not closely linked to biosynthetic genes. Instead it is located within a cluster of ribosomal protein operons.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: *Antibiotics, Peptide--pharmacology--PD; *Genes, Bacterial; *Methyltransferases--genetics--GE; *Streptomyces--genetics--GE; *Thiostrepton--pharmacology--PD; Amino Acid Sequence; Chromosome Mapping; Cloning, Molecular; Cosmids; Drug Resistance, Microbial--genetics--GE; Gene Library; Molecular Sequence Data; Multigene Family; **Mutagenesis , Insertional** ; Operon--genetics--GE; Peptide Synthases--genetics--GE; Ribosomal Proteins--genetics--GE; Sequence Analysis, DNA; Sequence Homology, Amino Acid

Molecular Sequence Databank No.: GENBANK/L39157; GENBANK/M17788; GENBANK/X17524; GENBANK/X54994; GENBANK/X67057; GENBANK/Z14314

CAS Registry No.: 0 (Antibiotics, Peptide); 0 (Cosmids); 0 (Ribosomal Proteins); 1393-48-2 (Thiostrepton)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.66 (rRNA (**adenosine** -O-2'-) **methyltransferase**); EC 6.3.2. (Peptide Synthases)

Record Date Created: 19951212

Record Date Completed: 19951212

8/9/21

12685539 PMID: 7608082

Incomplete entry of bacteriophage T7 DNA into F plasmid **-containing Escherichia coli.**

Garcia L R; Molineux I J

Department of Microbiology, University of Texas, Austin 78712-1095, USA.

Journal of bacteriology (UNITED STATES) Jul 1995, 177 (14) p4077-83, ISSN 0021-9193 Journal Code: 2985120R

Contract/Grant No.: GM32095; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The penetration of bacteriophage T7 DNA into F **plasmid** -containing Escherichia coli cells was determined by measuring Dam methylation of the entering genome. T7 strains that cannot productively infect F-containing cells fail to completely translocate their DNA into the cell before the

infection aborts. The entry of the first 44% of the genome occurs normally in an F-containing cell, but the entry of the remainder is aberrant. Bypassing the normal mode of entry of the T7 genome by transfecting naked DNA into competent cells fails to suppress F exclusion of phage development. However, overexpression of various nontoxic T7 1.2 alleles from a high-copy-number **plasmid** or expression of T3 1.2 from a T7 genome allows phage growth in the presence of F.

Tags: Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.

Descriptors: *Bacteriophage T7--growth and development--GD; *DNA, Viral --metabolism--ME; *Escherichia coli--virology--VI; *F Factor; Bacteriophage T7--genetics--GE; DNA, Viral--genetics--GE; Gene Expression; Genes, Viral; Genome, Viral; Methylation; **Mutation** ; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME; Transfection

CAS Registry No.: 0 (DNA, Viral); 0 (F Factor)

Enzyme No.: EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19950815

Record Date Completed: 19950815

8/9/22

DIALOG(R)File 155:MEDLINE(R)

12684915 PMID: 7607497

Expression of the SalI restriction-modification system of Streptomyces albus G in Escherichia coli.

Alvarez M A; Gomez A; Gomez P; Rodicio M R

Departamento de Biologia Funcional, Universidad de Oviedo, Spain.

Gene (NETHERLANDS) May 19 1995, 157 (1-2) p231-2, ISSN 0378-1119 Journal Code: 7706761

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The salIR and salIM genes of Streptomyces albus G encode the restriction endonuclease (ENase) and DNA methyltransferase (MTase) of the SalI restriction-modification (R-M) system. In S. albus G, the genes constitute an operon that is mainly transcribed from a promoter located upstream from salIR, the first gene of the operon. In addition, a second promoter, at the 3' end of salIR, allows independent transcription of the MTase gene. Expression of salIR and salIM in Escherichia coli was investigated. The ENase gene was not expressed in the **heterologous** host, probably due to inactivity of the main promoter of the salI operon. In contrast to salIR, salIM was functional in E. coli. Preliminary S1 nuclease mapping experiments suggest that the **alternative** promoter of the MTase gene can initiate transcription in the **heterologous** , as well as in the homologous host.

Tags: Support, Non-U.S. Gov't

Descriptors: Deoxyribonucleases, Type II Site-Specific--biosynthesis--BI; *Recombinant Proteins--biosynthesis--BI; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--biosynthesis--BI; *Streptomyces--enzymology--EN ; Cloning, Molecular--methods--MT; Deoxyribonucleases, Type II Site-Specific--genetics--GE; Escherichia coli--metabolism--ME; Gene Expression; Genes, Bacterial; Operon; Promoter Regions (Genetics); Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Streptomyces--genetics--GE

CAS Registry No.: 0 (Recombinant Proteins)

Enzyme No.: EC 2.1.1.- (DNA modification methylase SalGI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (endodeoxyribonuclease SalI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)

Gene Symbol: salIM; salIR

Record Date Created: 19950811

Record Date Completed: 19950811

8/9/23
DIALOG(R)File 155:MEDLINE(R)

12680835 PMID: 7603433
Characterization of three genes in the dam-containing operon of Escherichia coli.
Lyngstadaas A; Lobner-Olesen A; Boye E
Department of Biophysics, Institute for Cancer Research, Montebello, Oslo, Norway.
Molecular & general genetics - MGG (GERMANY) Jun 10 1995, 247 (5) p546-54, ISSN 0026-8925 Journal Code: 0125036
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The dam-containing operon in Escherichia coli is located at 74 min on the chromosomal map and contains the genes aroK, aroB, a gene called urf74.3, dam and trpS. We have determined the nucleotide sequence between the dam and trpS genes and show that it encodes two proteins with molecular weights of 24 and 27 kDa. Furthermore, we characterize the three genes urf74.3, 24kDa, 27kDa and the proteins they encode. The predicted amino acid sequences of the 24 and 27 kDa proteins are similar to those of the CbbE and CbbZ proteins, respectively, of the Alcaligenes eutrophus cbb operon, which encodes enzymes involved in the Calvin cycle. In separate experiments, we have shown that the 24 kDa protein has d-ribulose-5-phosphate epimerase activity (similar to CbbE), and we call the gene rpe. Similarly, the 27 kDa protein has 2-phosphoglycolate phosphatase activity (similar to CbbZ), and we name the gene gph. The Urf74.3 protein, with a predicted molecular weight of 46 kDa, migrated as a 70 kDa product under denaturing conditions. Overexpression of Urf74.3 induced cell filamentation, indicating that Urf74.3 directly or indirectly interferes with cell division. We present evidence for translational coupling between aroB and urf74.3 and also between rpe and gph. Proteins encoded in the dam superoperon appear to be largely unrelated: Dam, and perhaps Urf74.3, are involved in cell cycle regulation, AroK, AroB, and TrpS function in aromatic amino acid biosynthesis, whereas Rpe and Gph are involved in carbohydrate metabolism.
Tags: Support, Non-U.S. Gov't
Descriptors: Escherichia coli--genetics--GE; *Genes, Bacterial--genetics --GE; *Methyltransferases--genetics--GE; *Site-Specific DNA- **Methyltransfer ase** (**Adenine** -Specific); Amino Acid Sequence; Bacterial Proteins --biosynthesis--BI; Bacterial Proteins--genetics--GE; Base Sequence; Chromosome Mapping; Escherichia coli--enzymology--EN; Molecular Sequence Data; **Mutagenesis** , **Insertional** ; Operon--genetics--GE
Molecular Sequence Databank No.: GENBANK/Z19601
CAS Registry No.: 0 (Bacterial Proteins)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Gene Symbol: aroB; aroK; dam; trpS; urf74.3
Record Date Created: 19950809
Record Date Completed: 19950809

8/9/24
DIALOG(R)File 155:MEDLINE(R)

12593736 PMID: 7707369
Selection of a remote cleavage site by I-tevI, the td intron-encoded endonuclease.
Bryk M; Belisle M; Mueller J E; Belfort M
Molecular Genetics Program Wadsworth Center, State University of New York, New York State Department of Health, Albany 12201-0509, USA.

chromatin structure.
Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Descriptors: Fungal Proteins--biosynthesis--BI; *Gene Expression Regulation, Fungal; *Genes, Fungal; *Saccharomyces cerevisiae--metabolism --ME; *Saccharomyces cerevisiae Proteins; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); *Transcription Factors --biosynthesis--BI; *Transcription, Genetic; Base Sequence; Chromatography, Gel; Chromatography, High Pressure Liquid; DNA Primers; Fungal Proteins --genetics--GE; Fungal Proteins--metabolism--ME; Genotype; Methyltransfera ses--metabolism--ME; Molecular Sequence Data; **Plasmids** ; Polymerase Chain Reaction; Promoter Regions (Genetics); Recombinant Fusion Proteins --biosynthesis--BI; Saccharomyces cerevisiae--genetics--GE; Sequence Deletion; Suppression, Genetic
CAS Registry No.: 0 (DNA Primers); 0 (Fungal Proteins); 0 (Hpr1 protein, S cerevisiae); 0 (Plasmids); 0 (Recombinant Fusion Proteins); 0 (Saccharomyces cerevisiae Proteins); 0 (Transcription Factors)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Gene Symbol: ADH1; GAL1; HO; HPR1; SNF2; SNF5; SNF6; SUC2; SWI1; SWI2; SWI3; hpr1
Record Date Created: 19950323
Record Date Completed: 19950323

8/9/27
DIALOG(R)File 155:MEDLINE(R)

12518929 PMID: 7528201
In vivo restriction by LlaI is encoded by three genes, arranged in an operon with llaIM, on the conjugative Lactococcus plasmid **pTR2030.**
O'Sullivan D J; Zagula K; Klaenhammer T R
Department of Food Science, North Carolina State University, Raleigh 27695-7624.
Journal of bacteriology (UNITED STATES) Jan 1995, 177 (1) p134-43, ISSN 0021-9193 Journal Code: 2985120R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The LlaI restriction and modification (R/M) system is encoded on pTR2030, a 46.2-kb conjugative **plasmid** from Lactococcus lactis. The llaI methylase gene, sequenced previously, encodes a functional type IIS methylase and is located approximately 5 kb upstream from the abiA gene, encoding abortive phage resistance. In this study, the sequence of the region between llaIM and abiA was determined and revealed four consecutive open reading frames (ORFs). Northern (RNA) analysis showed that the four ORFs were part of a 7-kb operon with llaIM and the downstream abiA gene on a separate transcriptional unit. The deduced protein sequence of ORF2 revealed a P-loop consensus motif for ATP/GTP-binding sites and a three-part consensus motif for GTP-binding proteins. Data bank searches with the deduced protein sequences for all four ORFs revealed no homology except for ORF2 with MerB, in three regions that coincided with the GTP-binding motifs in both proteins. To phenotypically analyze the llaI operon, a 9.0-kb fragment was cloned into a high-copy-number lactococcal shuttle **vector** , pTRKH2. The resulting construct, pTRK370, exhibited a significantly higher level of in vivo restriction and modification in L. lactis NCK203 than the low-copy-number parental **plasmid** , pTR2030. A combination of deletion constructions and frameshift **mutations** indicated that the first three ORFs were involved in LlaI restriction, and they were therefore designated llaI.1, llaI.2, and llaI.3. Mutating llaI.1 completely abolished restriction, while disrupting llaI.2 or llaI.3 allowed an inefficient restriction of phage DNA to occur, manifested primarily by a variable plaque phenotype. ORF4 had no discernible effect on in vivo restriction. A frameshift **mutation** in llaIM proved lethal to L. lactis NCK203, implying

that the restriction component was active without the modification subunit. These results suggested that the LlaI R/M system is unlike any other R/M system studied to date and has diverged from the type IIS class of restriction enzymes by acquiring some characteristics reminiscent of type I enzymes.

Tags: Comparative Study; Support, Non-U.S. Gov't

Descriptors: Genes, Bacterial--genetics--GE; *Lactococcus lactis --genetics--GE; *Operon--genetics--GE; * **Plasmids** --genetics--GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Amino Acid Sequence; Base Sequence; Blotting, Northern; Conjugation, Genetic; Conserved Sequence; DNA **Mutational** Analysis; DNA Restriction-Modification Enzymes--classification--CL; DNA Restriction-Modification Enzymes--genetics--GE; Frameshift **Mutation** ; Molecular Sequence Data; Open Reading Frames--genetics--GE; RNA, Bacterial--genetics--GE; Sequence Analysis, DNA; Sequence Homology, Amino Acid

Molecular Sequence Databank No.: GENBANK/U17233

CAS Registry No.: 0 (DNA Restriction-Modification Enzymes); 0 (Plasmids); 0 (RNA, Bacterial)

Enzyme No.: EC 2.1.1.- (DNA modification methylase LlaI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Gene Symbol: abiA; llaI; llaM

Record Date Created: 19950125

Record Date Completed: 19950125

8/9/28

DIALOG(R)File 155:MEDLINE(R)

10414043 PMID: 8549991

Overproduction of the Hsd subunits leads to the loss **of temperature-sensitive restriction and modification phenotype.**

Weiserova M; Janscak P; Zinkevich V; Hubacek J

Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague.

Folia microbiologica (CZECH REPUBLIC) 1994, 39 (6) p452-8, ISSN 0015-5632 Journal Code: 0376757

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The genes hsdM and hsdS for M. EcoKI modification methyltransferase and the complete set of hsdR, hsdM and hsdS genes coding for R. EcoKI restriction endonuclease, both with and without a temperature-sensitive (ts) **mutation** in hsdS gene, were cloned in pBR322 **plasmid** and introduced into E. coli C (a strain without a natural restriction-modification (R-M) system). The strains producing only the methyltransferase, or together with the endonuclease, were thus obtained. The hsdSts-1 **mutation** , mapped previously in the distal variable region of the hsdS gene with C1 245-T transition has no effect on the R-M phenotype expressed from cloned genes in bacteria grown at 42 degrees C. In clones **transformed** with the whole hsd region an alleviation of R-M functions was observed immediately after the **transformation** , but after subculture the **transformants** expressed the wild-type R-M phenotype irrespective of whether the wild-type or the **mutant** hsdS allele was present in the hybrid **plasmid** . Simultaneous overproduction of HsdS and HsdM subunits impairs the ts effect of the hsdSts-1 **mutation** on restriction and modification.

Tags: Support, Non-U.S. Gov't

Descriptors: DNA Restriction Enzymes--biosynthesis--BI; *Escherichia coli --enzymology--EN; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--biosynthesis--BI; DNA Restriction Enzymes--genetics--GE; DNA Restriction Enzymes--physiology--PH; Escherichia coli--genetics--GE; Genes, Structural, Bacterial--genetics--GE; **Mutation** ; Phenotype; **Plasmids** --analysis--AN; **Plasmids** --genetics--GE; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--physiology--PH; Temperature;

Transformation , Bacterial
CAS Registry No.: 0 (Plasmids)
Enzyme No.: EC 2.1.1.- (DNA modification methylase EcoKI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21 (DNA Restriction Enzymes); EC 3.1.21.- (endodeoxyribonuclease EcoKI)
Record Date Created: 19960220
Record Date Completed: 19960220

8/9/29
DIALOG(R)File 155:MEDLINE(R)

10333332 PMID: 7828859
Site-directed mutagenesis **of double-stranded DNA by the polymerase chain reaction.**
Weiner M P; Costa G L; Schoettlin W; Cline J; Mathur E; Bauer J C
Stratagene Cloning Systems, La Jolla, CA 92037.
Gene (NETHERLANDS) Dec 30 1994, 151 (1-2) p119-23, ISSN 0378-1119 Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
We have developed a facile procedure for rapid PCR-based site-directed **mutagenesis** of double-stranded DNA. Increasing the initial template concentration and decreasing the PCR cycles to 5-10 allows us to **reduce** the rate of undesired second-site **mutations** and dramatically increase the time savings. Following PCR, DpnI treatment is used to select against parental DNA molecules. The DpnI (target sequence 5'-Gm6ATC) is specific for methylated and hemimethylated DNA and is used to digest parental DNA and select for **mutation** -containing amplified DNA. DNA isolated from almost all common Escherichia coli strains is Dam methylated and therefore susceptible to DpnI digestion. Pfu DNA polymerase is used, prior to intramolecular ligation of the linear template, to remove any bases extended onto the 3' ends of the PCR product by Taq DNA polymerase. The recircularized **vector** DNA incorporating the desired **mutations** is **transformed** into E. coli. This method can be used independently of any host strain and **vector** .
Tags: Comparative Study
Descriptors: DNA--chemistry--CH; * **Mutagenesis** , Site-Directed; *Polymerase Chain Reaction--methods--MT; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Base Sequence; DNA--genetics--GE; DNA Primers; Deoxyribonucleases, Type II Site-Specific; Escherichia coli --genetics--GE; Indicators and Reagents; Methyltransferases; Molecular Sequence Data; Phenotype
CAS Registry No.: 0 (DNA Primers); 0 (Indicators and Reagents); 9007-49-2 (DNA)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (endodeoxyribonuclease DpnI); EC 3.1.21.- (endodeoxyribonuclease MboI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)
Record Date Created: 19950222
Record Date Completed: 19950222

8/9/30
DIALOG(R)File 155:MEDLINE(R)

10231961 PMID: 7934887
Novel growth rate control of dam gene expression in Escherichia coli.
Rasmussen L J; Marinus M G; Lobner-Olesen A
Department of Pharmacology, University of Massachusetts Medical School, Worcester 01655.

Molecular microbiology (ENGLAND) May 1994, 12 (4) p631-8, ISSN 0950-382X Journal Code: 8712028
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Transcription of the dam gene in Escherichia coli is growth rate regulated by a mechanism distinct from that used for ribosomal RNA gene promoters. Single-copy operon fusions to lacZ indicated that the major promoter, P2, is responsible for most or all of the growth rate dependence. Promoter P2 is a typical sigma 70 promoter with 18 bp spacing between the -10 and -35 hexamers. Primer extension analysis was used to show that there was no inhibition of transcription from promoter P2 in cells induced for the stringent response. Beta-galactosidase specific activity from a single-copy dam::lacZ fusion was unaffected by either excess rrnB RNA or the level of Fis protein. Thus growth rate control of dam gene expression differs from that of the rRNA and tRNA genes by its lack of response to stringent control, ribosomal feedback and enhanced transcription by Fis protein. We devised a procedure for selection of **mutant** cells in which dam gene expression was unregulated. One such **mutant** (cde-4), obtained by miniTn10 **insertion** , showed the same level of beta-galactosidase activity at all growth rates tested. In contrast, growth rate-dependent expression of the rrnB gene was unaffected by cde-4 confirming the different modes of regulation. The cde-4::miniTn10 **insertion** is located close to kilobase 670 on the physical map in or near the lipB gene.

Tags: Support, Non-U.S. Gov't

Descriptors: Escherichia coli--genetics--GE; *Escherichia coli Proteins; *Genes, Bacterial; *Methyltransferases--genetics--GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Base Sequence; **Carrier** Proteins --genetics--GE; Cell Division--genetics--GE; Chromosome Mapping; DNA, Bacterial--genetics--GE; Escherichia coli--enzymology--EN; Escherichia coli--growth and development--GD; Factor For Inversion Stimulation Protein; Feedback; Gene Expression Regulation, Bacterial; Integration Host Factors; Molecular Sequence Data; **Mutation** ; Promoter Regions (Genetics); Ribosomes --metabolism--ME; Transcription, Genetic

CAS Registry No.: 0 (Carrier Proteins); 0 (DNA, Bacterial); 0 (Escherichia coli Proteins); 0 (Factor For Inversion Stimulation Protein) ; 0 (Integration Host Factors); 0 (integration host factor, E coli)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Gene Symbol: cde-4; dam

Record Date Created: 19941101

Record Date Completed: 19941101

8/9/31

DIALOG(R)File 155:MEDLINE(R)

10221397 PMID: 7925357

Overexpression of the thiostrepton-resistance gene from Streptomyces azureus in Escherichia coli and characterization of recognition sites of the 23S rRNA A1067 2'-methyltransferase in the guanosine triphosphatase center of 23S ribosomal RNA.

Bechthold A; Floss H G

Department of Chemistry, University of Washington, Seattle 98195.

European journal of biochemistry / FEBS (GERMANY) Sep 1 1994, 224 (2) p431-7, ISSN 0014-2956 Journal Code: 0107600

Contract/Grant No.: AI-20264; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The thiostrepton-resistance gene encoding the 23S rRNA A1067

methyltransferase from Streptomyces azureus has been overexpressed in Escherichia coli using a T7-RNA-polymerase-dependent expression **vector**. The protein was efficiently expressed at levels up to 20% of total soluble protein and purified to near homogeneity. Kinetic parameters for S-adenosyl-L-methionine (Km = 0.1 mM) and an RNA fragment containing nucleotides 1029-1122 of the 23S ribosomal RNA from E. coli (Km = 0.001 mM) were determined. S-Adenosyl-L-homocysteine showed competitive product inhibition (Ki = 0.013 mM). Binding of either thiostrepton or protein L11 inhibited methylation. RNA sequence variants of the RNA fragment with **mutations** in nucleotides 1051-1108 were tested as substrates for the methylase. The experimental data indicate that methylation is dependent on the secondary structure of the hairpin including nucleotide A1067 and the exact sequence U(1066)-A(1067)-G(1068)-A(1069)-A(1070) of the single strand.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Drug Resistance, Microbial--genetics--GE; *GTP Phosphohydrolases--metabolism--ME; *Methyltransferases--metabolism--ME; *RNA, Ribosomal, 23S--metabolism--ME; *Streptomyces--genetics--GE; *Thiostrepton--pharmacology--PD; Base Sequence; Binding Sites; Binding, Competitive; Cloning, Molecular; DNA Primers; Escherichia coli; Gene Expression; Genes, Bacterial; Kinetics; Methyltransferases--biosynthesis --BI; Methyltransferases--isolation and purification--IP; Molecular Sequence Data; Molecular Weight; Nucleic Acid Conformation; **Plasmids**; Polymerase Chain Reaction; Recombinant Proteins--metabolism--ME; S-Adenosylhomocysteine--pharmacology--PD; Streptomyces--drug effects--DE; Substrate Specificity

CAS Registry No.: 0 (DNA Primers); 0 (Plasmids); 0 (RNA, Ribosomal, 23S); 0 (Recombinant Proteins); 1393-48-2 (Thiostrepton); 979-92-0 (S-Adenosylhomocysteine)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.66 (rRNA (**adenosine** -O-2'-) **methyltransferase**); EC 3.6.1.- (GTP Phosphohydrolases)

Record Date Created: 19941027

Record Date Completed: 19941027

8/9/32

DIALOG(R)File 155:MEDLINE(R)

10092818 PMID: 8200522

The dam and dcm strains of Escherichia coli--a review.

Palmer B R; Marinus M G

Department of Plant and Microbial Sciences, University of Canterbury, Christchurch, New Zealand.

Gene (NETHERLANDS) May 27 1994, 143 (1) p1-12, ISSN 0378-1119 Journal Code: 7706761

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The construction of a variety of strains deficient in the methylation of adenine and cytosine residues in DNA by the methyltransferases (MTases) Dam and Dcm has allowed the study of the role of these enzymes in the biology of Escherichia coli. Dam methylation has been shown to play a role in coordinating DNA replication initiation, DNA mismatch repair and the regulation of expression of some genes. The regulation of expression of dam has been found to be complex and influenced by five promoters. A role for Dcm methylation in the cell remains elusive and dcm- cells have no obvious phenotype. dam- and dcm- strains have a range of uses in molecular biology and bacterial genetics, including preparation of DNA for restriction by some restriction endonucleases, for **transformation** into other bacterial species, nucleotide sequencing and site-directed **mutagenesis**. A variety of assays are available for rapid detection of both the Dam and Dcm phenotypes. A number of restriction systems in E. coli have been described which recognise **foreign** DNA methylation, but ignore Dam and Dcm methylation. Here, we describe the most commonly used **mutant** alleles of

dam and dcm and the characteristics of a variety of the strains that **carry** these genes. A description of several **plasmids** that **carry** dam gene constructs is also included. (78 Refs.)

Tags: Support, Non-U.S. Gov't

Descriptors: DNA (Cytosine-5-)-Methyltransferase--genetics--GE; *DNA, Bacterial--metabolism--ME; *Escherichia coli--genetics--GE; *Genes, Structural, Bacterial; *Methyltransferases--genetics--GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Alleles; Cloning, Molecular; DNA Repair; Escherichia coli--classification--CL; Escherichia coli --enzymology--EN; Gene Expression Regulation, Enzymologic; Methylation; **Mutation** ; **Plasmids**

CAS Registry No.: 0 (DNA, Bacterial); 0 (Plasmids)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.37 (DNA (Cytosine-5-)-Methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Gene Symbol: dam; dcm

Record Date Created: 19940706

Record Date Completed: 19940706

8/9/33

DIALOG(R)File 155:MEDLINE(R)

10088373 PMID: 8196622

The yeast GAL11 protein is involved in regulation of the structure and the position effect of telomeres.

Suzuki Y; Nishizawa M

Department of Microbiology, Keio University School of Medicine, Tokyo, Japan.

Molecular and cellular biology (UNITED STATES) Jun 1994, 14 (6) p3791-9, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

GAL11 is an auxiliary transcription factor that functions either positively or negatively, depending on the structure of the target promoters and the combination of DNA-bound activators. In this report, we demonstrate that a gal11 delta **mutation** caused a decrease in the length of the telomere C1-3A tract, a derepression of URA3 when it is placed next to telomere, and an increase in accessibility of the telomeric region to dam methylase, indicating that GAL11 is involved in the regulation of the structure and the position effect of telomeres. The defective position effect in a gal11 delta strain was suppressed by overproduction of SIR3, whereas overexpression of GAL11 failed to restore the telomere position effect in a sir3 delta strain. Hyperproduced GAL11 could partially suppress the defect in silencing at HMR in a sir1 delta **mutant** but not that in a sir3 delta **mutant** , suggesting that GAL11 can replace SIR1 function partly in the silencing of HMR. Overproduced SIR3 also could restore silencing at HMR in sir1 delta cells. In contrast, SIR1 in a multicopy **plasmid** relieved the telomere position effect, especially in a gal11 delta **mutant** . Since chromatin structure is thought to play a major role in the silencing at both the HM loci and telomeres, GAL11 is likely to participate in the regional regulation of transcription by the HM loci and telomeres, GAL11 is likely to participate in the regional regulation of transcription by modulating the chromatin structure.

Tags: Support, Non-U.S. Gov't

Descriptors: Fungal Proteins--genetics--GE; *Fungal Proteins--metabolism --ME; *Saccharomyces cerevisiae--metabolism--ME; *Saccharomyces cerevisiae Proteins; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); *Telomere--physiology--PH; *Trans-Activators; *Transcription Factors --genetics--GE; *Transcription Factors--metabolism--ME; Cloning, Molecular ; DNA, Fungal--metabolism--ME; Escherichia coli; Fungal Proteins --biosynthesis--BI; Gene Expression Regulation, Fungal; Genes, Fungal; Genotype; Methylation; Methyltransferases--metabolism--ME; **Mutagenesis** ;

Promoter Regions (Genetics); Restriction Mapping; Saccharomyces cerevisiae --genetics--GE; Saccharomyces cerevisiae--physiology--PH; Transcription Factors--biosynthesis--BI

CAS Registry No.: 0 (DNA, Fungal); 0 (Fungal Proteins); 0 (Gal11 protein, S cerevisiae); 0 (Saccharomyces cerevisiae Proteins); 0 (Trans-Activators); 0 (Transcription Factors)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Gene Symbol: GAL11; HMR

Record Date Created: 19940624

Record Date Completed: 19940624

8/9/34

DIALOG(R)File 155:MEDLINE(R)

10050004 PMID: 8163534

A novel class of FokI restriction endonuclease mutants **that cleave hemi-methylated substrates.**

Waugh D S; Sauer R T

Department of Biology, Massachusetts Institute of Technology, Cambridge 02139.

Journal of biological chemistry (UNITED STATES) Apr 22 1994, 269 (16) p12298-303, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: AI-16892; AI; NIAID; GM-13909; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A genetic screen was used to identify amino acid substitutions that enable the FokI restriction endonuclease to cleave DNA in cells that express the cognate methyltransferase activity. Missense **mutations** that give rise to this phenotype were isolated at eight different positions (G188K, P196S, T343I, S388N, S395F, E407K, E410K, D421N), clustered in two regions of the polypeptide sequence of FokI. Two of the **mutant** endonucleases (P196S and D421N) were purified to homogeneity and analyzed in detail. Both **mutants** cleave FokI target sites (5'-GGATG-3') in a manner similar to the wild-type enzyme. Neither **mutant** cleaved noncanonical sequences, but both efficiently cleaved DNA substrates containing hemi-methylated FokI sites. This class of **mutations** has not been observed with other restriction enzymes.

Tags: Comparative Study; Support, U.S. Gov't, P.H.S.

Descriptors: Deoxyribonucleases, Type II Site-Specific--metabolism--ME; *Flavobacterium--enzymology--EN; * **Plasmids** --metabolism--ME; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME ; Base Sequence; Cloning, Molecular; Deoxyribonucleases, Type II Site-Specific--biosynthesis--BI; Escherichia coli; Flavobacterium--genetics --GE; Hydroxylamine; Hydroxylamines--toxicity--TO; Methylation; Molecular Sequence Data; **Mutagenesis** ; Point **Mutation** ; Restriction Mapping; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--biosynthesis --BI; Substrate Specificity

CAS Registry No.: 0 (Hydroxylamines); 0 (Plasmids); 7803-49-8 (Hydroxylamine)

Enzyme No.: EC 2.1.1.- (DNA modification methylase FokI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (endodeoxyribonuclease FokI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)

Gene Symbol: dinD; lacI; lacZ

Record Date Created: 19940526

Record Date Completed: 19940526

8/9/35

DIALOG(R)File 155:MEDLINE(R)

methyltransferase. The location of the genes on the clone was determined and this information was used to construct a small deletion (400 bp) that results in an R-M+ phenotype. This **mutation** was recombined onto the Y. enterocolitica chromosome to give an R-M+ **mutant** which showed at least a 1000-fold increase in electroporation frequency compared to the wild-type strain. Southern analysis using a probe derived from yenIMR indicated that American serotype strains have this locus whereas non-American serotype strains do not.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: Deoxyribonucleases, Type II Site-Specific--genetics--GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; * **Transformation** , Genetic; *Yersinia enterocolitica--enzymology--EN; Chromosome Mapping; Cloning, Molecular; **Mutation** ; Phenotype; Virulence --genetics--GE; Yersinia enterocolitica--pathogenicity--PY

Enzyme No.: EC 2.1.1.- (DNA modification methylase PstI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (endodeoxyribonuclease PstI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)

Gene Symbol: yenIMR

Record Date Created: 19940301

Record Date Completed: 19940301

8/9/38

DIALOG(R)File 155:MEDLINE(R)

09909170 PMID: 8253669

Evidence of two levels of control of P1 oriR and host oriC replication origins by DNA adenine methylation.

Abeles A; Brendler T; Austin S

Laboratory of Chromosome Biology, National Cancer Institute-Frederick Cancer Research and Development Center, Maryland 21701-1201.

Journal of bacteriology (UNITED STATES) Dec 1993, 175 (24) p7801-7, ISSN 0021-9193 Journal Code: 2985120R

Contract/Grant No.: NO1-CO-74101; CO; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A **mutant** mini-P1 **plasmid** with increased copy number can be established in Dam- strains of Escherichia coli, where mini-P1 **plasmid** replication is normally blocked. Comparison of this **plasmid** and a **plasmid** driven by the host oriC replication origin showed that both origins are subject to control by methylation at two different levels. First, both origins appear to be subject to **negative** regulation acting at the level of hemimethylation. This probably involves the sequestration of the hemimethylated DNA produced by replication, as has been previously described for oriC. Second, both origins show a positive requirement for adenine methylation for efficient function in vivo. This conclusion is supported by the behavior of the P1 origin in an improved in vitro replication system. In vitro, where sequestration of hemimethylated DNA is not expected to occur, the hemimethylated P1 origin DNA was fully functional as a template. However, the activity of fully unmethylated DNA was severely restricted in comparison with that of either of the methylated forms. This in vitro uncoupling of the two effects of origin methylation suggests that two separate mechanisms are involved.

Tags: Comparative Study; Support, U.S. Gov't, P.H.S.

Descriptors: Adenine--metabolism--ME; *DNA Replication; *DNA, Bacterial --biosynthesis--BI; *Escherichia coli--genetics--GE; * **Plasmids** ; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME ; Bacteriophage T4--enzymology--EN; Blotting, Southern; Escherichia coli --metabolism--ME; Methylation; **Mutagenesis** ; Repetitive Sequences, Nucleic Acid; Templates, Genetic; **Transformation** , Genetic

CAS Registry No.: 0 (DNA, Bacterial); 0 (Plasmids); 73-24-5 (Adenine)

Enzyme No.: EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19940110
Record Date Completed: 19940110

8/9/39

DIALOG(R)File 155:MEDLINE(R)

09873112 PMID: 8223468

Macroevolution by transposition: drastic modification of DNA recognition by a type I restriction enzyme following Tn5 transposition.

Meister J; MacWilliams M; Hubner P; Jutte H; Skrzypek E; Piekarowicz A; Bickle T A
Department of Microbiology, Biozentrum, Basel University, Switzerland.

EMBO journal (ENGLAND) Dec 1993, 12 (12) p4585-91, ISSN 0261-4189 Journal Code: 8208664
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

We have characterized a novel **mutant** of EcoDXXI, a type IC DNA restriction and modification (R-M) system, in which the specificity has been **altered** due to a Tn5 **insertion** into the middle of hsdS, the gene which encodes the polypeptide that confers DNA sequence specificity to both the restriction and the modification reactions. Like other type I enzymes, the wild type EcoDXXI recognizes a sequence composed of two asymmetrical half sites separated by a spacer region: TCA(N7)RTTC. Purification of the EcoDXXI **mutant** methylase and subsequent in vitro DNA methylation assays identified the **mutant** recognition sequence as an interrupted palindrome, TCA(N8)TGA, in which the 5' half site of the wild type site is repeated in inverse orientation. The additional base pair in the non-specific spacer of the **mutant** recognition sequence maintains the proper spacing between the two methylatable adenine groups. Sequencing of both the wild type and **mutant** EcoDXXI hsdS genes showed that the Tn5 **insertion** occurred at nucleotide 673 of the 1221 bp gene. This effectively deletes the entire carboxyl-terminal DNA binding domain which recognizes the 3' half of the EcoDXXI binding site. The truncated hsdS gene still encodes both the amino-terminal DNA binding domain and the conserved repeated sequence that defines the length of the recognition site spacer region. We propose that the EcoDXXI **mutant** methylase utilizes two truncated hsdS subunits to recognize its binding site. The implications of this finding in terms of subunit interactions and the malleability of the type I R-M systems will be discussed.

Tags: Support, Non-U.S. Gov't
Descriptors: *DNA--metabolism--ME; *DNA Restriction-Modification Enzymes --metabolism--ME; *DNA Transposable Elements; *Deoxyribonucleases, Type I Site-Specific--metabolism--ME; *Evolution; Base Sequence; DNA--genetics--GE ; DNA Restriction-Modification Enzymes--genetics--GE; Deoxyribonucleases, Type I Site-Specific--genetics--GE; Escherichia coli; Molecular Sequence Data; **Mutagenesis** ; **Plasmids** ; Restriction Mapping; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME; Substrate Specificity
CAS Registry No.: 0 (DNA Restriction-Modification Enzymes); 0 (DNA Transposable Elements); 0 (Plasmids); 9007-49-2 (DNA)
Enzyme No.: EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (endodeoxyribonuclease EcoDXXI); EC 3.1.21.3 (Deoxyribonucleases, Type I Site-Specific)
Gene Symbol: hsdS
Record Date Created: 19931221
Record Date Completed: 19931221

8/9/40

DIALOG(R)File 155:MEDLINE(R)

09820433 PMID: 8371109

pRJ5: a naturally occurring Staphylococcus aureus plasmid **expressing constitutive macrolide-lincosamide-streptogramin B resistance contains a tandem duplication in the leader region of the ermC gene.**

Oliveira S S; Murphy E; Gamon M R; Bastos M C
Departamento de Microbiologia Geral, Cidade Universitaria, Rio de Janeiro, Brazil.

Journal of general microbiology (ENGLAND) Jul 1993, 139 (Pt 7) p1461-7, ISSN 0022-1287 Journal Code: 0375371
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The 2.55 kb Staphylococcus aureus **plasmid** , pRJ5, confers constitutive resistance to macrolide-lincosamide-streptogramin B (MLS) antibiotics. pRJ5 is nearly identical to the inducible MLS resistance **plasmid** pT48, and has homology with the S. aureus **plasmids** pE194 and pSN2. The HindIII-C and/or Hind-B fragments were required for stable maintenance of the **plasmid** and probably **carry** palA. **Plasmids** pRJ5 and pT48 were shown to belong to the same incompatibility group, Incl2 (L). DNA sequencing showed that pRJ5 contains a 28 bp direct tandem duplication in the leader/ **attenuator** region of ermC. This is likely to change the secondary structure of the methylase mRNA, allowing constitutive expression of ermC. The type of **mutation** found on **plasmid** pRJ5 is different from those observed in similar 2.5 kb constitutive MLS-resistance **plasmids** isolated from other Gram-positive bacteria, including staphylococci.

Tags: Support, Non-U.S. Gov't
Descriptors: Anti-Bacterial Agents--pharmacology--PD; *Macrolides; *Multigene Family; * **Plasmids** --genetics--GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; *Staphylococcus aureus--genetics--GE; Base Sequence; Drug Resistance, Microbial; Gene Expression Regulation, Bacterial; Genes, Bacterial--genetics--GE; Molecular Sequence Data; Nucleic Acid Conformation; Regulatory Sequences, Nucleic Acid--genetics--GE; Virginiamycin--pharmacology--PD
Molecular Sequence Databank No.: GENBANK/L04687
CAS Registry No.: 0 (Anti-Bacterial Agents); 0 (Macrolides); 0 (Plasmids); 11006-76-1 (Virginiamycin); 80738-43-8 (lincosamide)
Enzyme No.: EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19931008
Record Date Completed: 19931008

8/9/41

DIALOG(R)File 155:MEDLINE(R)

09795838 PMID: 8394707

Fully methylated oriC with negative **superhelicity forms an oriC-membrane complex before initiation of chromosome replication.**

Kataoka T; Wachi M; Nakamura J; Gayama S; Yamasaki M; Nagai K
Department of Bioengineering, Tokyo Institute of Technology, Kanagawa, Japan.

Biochemical and biophysical research communications (UNITED STATES) Aug 16 1993, 194 (3) p1420-6, ISSN 0006-291X Journal Code: 0372516
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

In an in vitro assay, the oriC DNA has been shown to bind to the outer membrane fraction only when it is hemimethylated (G.B. Ogden et al., Cell, 54, 127-135,1988). In this report, however, we demonstrated that a

significant amount of the oriC DNA was recovered from the cells just before initiation with the oriC DNA being fully methylated. Formation of this preinitiation oriC-membrane complex and following initiation of chromosome replication were strongly inhibited by novobiocin, a DNA gyrase B subunit inhibitor, which **reduced** the superhelicity of the reporter **plasmid** in the cells. On the other hand, both reactions proceeded in the presence of nalidixic acid, a DNA gyrase A subunit inhibitor, which did not have the effect of **reducing** the superhelicity. These results suggest that the **negative** superhelicity of the DNA is required for preinitiation oriC-membrane complex formation and following initiation event of replication.

Tags: Support, Non-U.S. Gov't

Descriptors: Cell Membrane--metabolism--ME; *DNA Replication; *DNA, Bacterial--metabolism--ME; *DNA, Superhelical--metabolism--ME; *Escherichia coli--genetics--GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Cell Compartmentation; Chromosomes, Bacterial; DNA Topoisomerases, Type II--antagonists and inhibitors--AI; Macromolecular Systems; Methylation; Methyltransferases; Nalidixic Acid--pharmacology--PD; Novobiocin--pharmacology--PD

CAS Registry No.: 0 (DNA, Bacterial); 0 (DNA, Superhelical); 0 (Macromolecular Systems); 303-81-1 (Novobiocin); 389-08-2 (Nalidixic Acid)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 5.99.1.3 (DNA Topoisomerases, Type II)

Record Date Created: 19930915

Record Date Completed: 19930915

8/9/42

DIALOG(R)File 155:MEDLINE(R)

09752326 PMID: 8320213

Repair of heteroduplex DNA molecules with multibase loops in Escherichia coli.

Carraway M; Marinus M G

Department of Pharmacology, University of Massachusetts Medical School, Worcester 01655.

Journal of bacteriology (UNITED STATES) Jul 1993, 175 (13) p3972-80, ISSN 0021-9193 Journal Code: 2985120R

Contract/Grant No.: GM33233; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The fate of heteroduplex molecules containing 5-, 7-, 9-, 192-, 410-, and 514-base loops after **transformation** of wild-type and various **mutant** strains of Escherichia coli has been examined. No evidence for repair was obtained for the wild type or for strains with **mutations** in the following genes: mutS, recA, recBC sbcBC, recD, recF, recJ, recN, recO, recR, recBC sbcBC recF uvrA, recG ruvC, ruvB, lexA3, lexA51, uvrA, nfo xth nth, polA(Ts), or pcnB. These results rule out the involvement of the SOS system and most known recombination and repair pathways. Repair of heteroduplex molecules containing 410- and 514-base loops was observed when a 1-base deletion- **insertion** mismatch was present nearby. The repair of both the mismatch and the loops was directed by the state of dam methylation of the DNA chains and was dependent on the product of the mutS gene. A high efficiency of repair (95%) was found even when the mismatch and the loops were 1,448 nucleotides apart. We conclude that multibase loops in DNA can be removed only as a consequence of corepair by dam-directed mismatch repair.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: DNA Repair; *DNA, Bacterial--metabolism--ME; *Escherichia coli--genetics--GE; *Nucleic Acid Heteroduplexes--metabolism--ME; *Polynucleotide Adenylyltransferase; *Site-Specific DNA- **Methyltransferase**

(**Adenine** -Specific); Bacterial Proteins--genetics--GE; Base Sequence; DNA, Single-Stranded--genetics--GE; Genes, Bacterial--genetics--GE; Genetic Markers--genetics--GE; Methyltransferases--metabolism--ME; Molecular Sequence Data; **Mutagenesis** , Site-Directed; Operator Regions (Genetics) --genetics--GE; **Plasmids** --genetics--GE; Recombination, Genetic; **Transformation** , Genetic

CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA, Bacterial); 0 (DNA, Single-Stranded); 0 (Genetic Markers); 0 (Nucleic Acid Heteroduplexes); 0 (Plasmids)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 2.7.7.- (pcnB protein); EC 2.7.7.19 (Polynucleotide Adenylyltransferase)

Gene Symbol: dam; mutS

Record Date Created: 19930730

Record Date Completed: 19930730

8/9/43

DIALOG(R)File 155:MEDLINE(R)

09649415 PMID: 8384292

Purification and DNA binding of the D protein, a putative resolvase of the F-factor of Escherichia coli.

Disque-Kochem C; Eichenlaub R

Universitat Bielefeld, Fakultat fur Biologie, Lehrstuhl fur Gentechnologie/Mikrobiologie, Bielefeld, FRG.

Molecular & general genetics - MGG (GERMANY) Feb 1993, 237 (1-2) p206-14, ISSN 0026-8925 Journal Code: 0125036

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The D protein encoded by **plasmid** mini-F promotes resolution of **plasmid** cointegrates or dimers of the F-factor or mini-F. In addition, two rfsF sequences are essential for this site-specific, recA-independent recombination event. The D gene was cloned into an expression **vector** and the gene product was overproduced in Escherichia coli and purified to homogeneity. The sequence of the N-terminus of the D protein was determined, thus permitting identification of the correct translational start codon in the nucleotide sequence that results in a 29.6 kDa protein. The binding site for the purified D protein is located within the mini-F NcoI-HpaI DNA fragment (192 bp). Binding seems to be affected by DNA methylation, since the protein did not bind to DNA isolated from a dam **mutant** of E. coli. The binding site, which is a region of approximately 28 bp and is located 160 bp downstream of the rfsF site, was identified by DNase I footprinting using fluorescence labelled DNA.

Tags: Support, Non-U.S. Gov't

Descriptors: DNA, Bacterial--metabolism--ME; *Escherichia coli --enzymology--EN; *F Factor--metabolism--ME; *Nucleotidyltransferases --metabolism--ME; *Recombination, Genetic; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Base Sequence; Cloning, Molecular ; Methyltransferases; Molecular Sequence Data; Nucleotidyltransferases --biosynthesis--BI; Nucleotidyltransferases--genetics--GE; Nucleotidyltra nsferases--isolation and purification--IP; Recombinant Proteins --biosynthesis--BI; Recombinant Proteins--isolation and purification--IP; Recombinant Proteins--metabolism--ME; Restriction Mapping; Sequence Analysis; Transposases

CAS Registry No.: 0 (DNA, Bacterial); 0 (F Factor); 0 (Recombinant Proteins)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 2.7.7 (Nucleotidyltransferases); EC 2.7.7.- (Transposases)

Record Date Created: 19930416

09469039 PMID: 1328813

Molecular cloning and characterization of two lincomycin-resistance genes, lmrA and lmrB, from Streptomyces lincolnensis 78-11.

Zhang H Z; Schmidt H; Piepersberg W
Bergische Universitat GH Wuppertal, Germany.

Molecular microbiology (ENGLAND) Aug 1992, 6 (15) p2147-57, ISSN 0950-382X Journal Code: 8712028
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Two different lincomycin-resistance determinants (lmrA and lmrB) from Streptomyces lincolnensis 78-11 were cloned in Streptomyces lividans 66 TK23. The gene lmrA was localized on a 2.16 kb fragment, the determined nucleotide sequence of which encoded a single open reading frame 1446 bp long. Analysis of the deduced amino acid sequence suggested the presence of 12 membrane-spanning domains and showed significant similarities to the methylenomycin-resistance protein (Mmr) from Streptomyces coelicolor, the QacA protein from Staphylococcus aureus, and several tetracycline-resistance proteins from both Gram-positive and Gram- **negative** bacteria, as well as to some sugar-transport proteins from Escherichia coli. The lmrB gene was actively expressed from a 2.7 kb fragment. An open reading frame of 837 bp could be localized which encoded a protein that was significantly similar to 23S rRNA **adenine** (2058)-N- **methyltransferases** conferring macrolide-lincosamide-streptogramin resistance. LmrB also had putative rRNA methyltransferase activity since lincomycin resistance of ribosomes was induced in lmrB-containing strains. Surprisingly, both enzymes, LmrA and LmrB, had a substrate specificity restricted to lincomycin and did not cause resistance to other lincosamides such as celesticetin and clindamycin, or to macrolides.

Tags: Comparative Study; Support, Non-U.S. Gov't
Descriptors: *Genes, Structural, Bacterial; *Lincomycin--pharmacology--PD ; *Streptomyces--genetics--GE; Amino Acid Sequence; **Carrier** Proteins --genetics--GE; Catalysis; Cloning, Molecular; DNA, Bacterial--isolation and purification--IP; Drug Resistance, Microbial--genetics--GE; Gene Expression Regulation, Bacterial; Methylation; Methyltransferases--genetics --GE; Molecular Sequence Data; Phenotype; Protons; RNA, Ribosomal, 23S --genetics--GE; Sequence Homology; Streptomyces--drug effects--DE
Molecular Sequence Databank No.: GENBANK/X59926; GENBANK/X62867
CAS Registry No.: 0 (Carrier Proteins); 0 (DNA, Bacterial); 0 (Protons); 0 (RNA, Ribosomal, 23S); 154-21-2 (Lincomycin)
Enzyme No.: EC 2.1.1. (Methyltransferases)
Gene Symbol: Mmr; QacA; lmrA; lmrB
Record Date Created: 19921026
Record Date Completed: 19921026

8/9/50

09438487 PMID: 1526989

Cofactor and DNA interactions in EcoRI DNA methyltransferase. Fluorescence spectroscopy and phenylalanine replacement for tryptophan 183.

Maegley K A; Gonzalez L; Smith D W; Reich N O
Department of Chemistry, University of California, Santa Barbara 93106.

Journal of biological chemistry (UNITED STATES) Sep 15 1992, 267 (26) p18527-32, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

EcoRI DNA methyltransferase contains tryptophans at positions 183 and

225. Tryptophan 225 is adjacent to residues previously implicated in S-adenosylmethionine (AdoMet) binding and to cysteine 223, previously shown to be the site of N-ethyl maleimide-mediated inactivation of the enzyme (Reich, N. O., and Everett, E. (1990) J. Biol. Chem. 265, 8929-8934; Everett, E. A., Falick, A. M., and Reich, N. O. (1990) J. Biol. Chem. 265, 17713-17719). The fluorescence spectra of the wild-type enzyme is centered at 338 nm indicating partial tryptophan solvent accessibility. Substitution of tryptophan 183 with phenylalanine results in a 45% drop in fluorescence intensity, but no shift in lambda max. DNA binding to the wild-type methyltransferase caused an increase in the fluorescence intensity, while binding to the tryptophan 183 **mutant** had a quenching effect, suggesting that DNA binding induces a conformational change near both tryptophans. Binding of AdoMet and various AdoMet analogs to the wild-type methyltransferase results in no change in the fluorescence spectrum when excitation occurs at 295 nm, suggesting that no conformational change occurs, and AdoMet does not interact with either tryptophan. In contrast, quenching was observed when excitation occurred at 280 nm, suggesting that AdoMet and its analogs may be quenching tyrosine to tryptophan energy transfer. Protein-ligand complexes were titrated with acrylamide, and the data also implicate conformational changes upon DNA binding but not upon AdoMet binding, consistent with previous limited proteolysis results (Reich, N. O., Maegley, K. A., Shoemaker, D.D., and Everett, E. (1991) Biochemistry 30, 2940-2946).

Tags: Support, U.S. Gov't, Non-P.H.S.

Descriptors: DNA--metabolism--ME; *Phenylalanine--genetics--GE; *S-Adenosylmethionine--metabolism--ME; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME; *Tryptophan--genetics--GE; Acrylamide; Acrylamides--chemistry--CH; Base Sequence; Fluorescence Polarization; Genetic **Vectors** ; Molecular Sequence Data; **Mutagenesis** , Site-Directed; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE

CAS Registry No.: 0 (Acrylamides); 0 (Genetic Vectors); 29908-03-0 (S-Adenosylmethionine); 63-91-2 (Phenylalanine); 73-22-3 (Tryptophan); 79-06-1 (Acrylamide); 9007-49-2 (DNA)

Enzyme No.: EC 2.1.1.- (DNA modification methylase EcoRI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19921019

Record Date Completed: 19921019

8/9/51

DIALOG(R)File 155:MEDLINE(R)

09431300 PMID: 1522064

Regulation of the macrolide-lincosamide-streptogramin B resistance gene ermD.

Hue K K; Bechhofer D H

Department of Biochemistry, Mount Sinai School of Medicine, New York, New York 10029.

Journal of bacteriology (UNITED STATES) Sep 1992, 174 (18) p5860-8, ISSN 0021-9193 Journal Code: 2985120R

Contract/Grant No.: AI-17472; AI; NIAID; GM-39516; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The erythromycin resistance gene ermD, which encodes an rRNA methylase protein, has an unusually long leader region (354 nucleotides). Previously, a single promoter-proximal leader peptide coding sequence was recognized from the nucleotide sequence, and erythromycin-induced ribosome stalling in this sequence was proposed to be required for the induction of methylase translation. We characterized spontaneously occurring and in vitro-constructed leader region **mutations** in an effort to understand the function of various segments of the long ermD leader region. A second leader peptide coding sequence was identified, and the location of **insertion** and point **mutations** that expressed ermD methylase

Enzyme No.: EC 2.1.1.- (DNA modification methylase EcoRI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19911224
Record Date Completed: 19911224

8/9/57

DIALOG(R)File 155:MEDLINE(R)

08875164 PMID: 2021649

Optically detected magnetic resonance study of the interaction of an arsenic(III) derivative of cacodylic acid with EcoRI methyl transferase.

Tsao D H; Maki A H
Department of Chemistry, University of California, Davis 95616.

Biochemistry (UNITED STATES) May 7 1991, 30 (18) p4565-72, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: ES-02622; ES; NIEHS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The interaction of the enzyme Escherichia coli RI methyl transferase (methylase) with an arsenic(III) derivative of cacodylic acid has been investigated by optical detection of triplet-state magnetic resonance (ODMR) spectroscopy in zero applied magnetic field. The reactive derivative (CH3)2AsSR is formed by the **reduction** of cacodylate by a thiol. The As(III) derivative binds to the enzyme by mercaptide exchange with a cysteine (Cys) residue located close to a tryptophan (Trp) site. The arsenical binding selectively induces an external heavy-atom effect, perturbing the nearby Trp residue in the enzyme. Zero-field splittings (ZFS) and total decay rate constants of the individual triplet-state sublevels of the Trp residue in the presence and absence of perturbation by As(III) have been determined. The perturbed Trp shows a large **reduction** in the overall decay lifetime compared with unperturbed Trp residue, exhibiting a high selectively for the Tx sublevel. This selectivity suggests that the As atom lies in the xz plane of the principal magnetic axis system of Trp, but not directly along the z (out-of-plane) axis. The accessibility of this enzyme binding site to the arsenical is decreased upon forming a ternary complex of methylase with sinefungin and a DNA oligomer, d[GCGAA(BrU)(BrU)CGC], containing two 5-bromouracil (BrU) bases in place of thymine within the hexadeoxynucleotide recognition sequence. This result indicates that the arsenical binding site in methylase which produces the Trp heavy-atom effect is protected from this ligand by ternary complex formation or the enzyme undergoes a conformation change, removing the Cys from the Trp site. This protection is also observed in fluorescence quenching experiments.(ABSTRACT TRUNCATED AT 250 WORDS)

Tags: Support, U.S. Gov't, P.H.S.
Descriptors: Arsenic--metabolism--ME; *Cacodylic Acid--metabolism--ME; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME ; Binding Sites; Bromouracil; **Carrier** Proteins--metabolism--ME; DNA --metabolism--ME; Dithiothreitol--metabolism--ME; Hydrolysis; Kinetics; Luminescence; Magnetic Resonance Spectroscopy; Mercaptoethanol--metabolism --ME; Methylation; Protein Conformation; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--chemistry--CH
CAS Registry No.: 0 (Carrier Proteins); 0 (folate-binding protein); 3483-12-3 (Dithiothreitol); 51-20-7 (Bromouracil); 60-24-2 (Mercaptoethanol); 7440-38-2 (Arsenic); 75-60-5 (Cacodylic Acid); 9007-49-2 (DNA)
Enzyme No.: EC 2.1.1.- (DNA modification methylase EcoRI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19910606
Record Date Completed: 19910606

8/9/58

DIALOG(R)File 155:MEDLINE(R)

08765790 PMID: 2176880

Fidelity of DNA recognition by the EcoRV restriction/modification system in vivo.

Taylor J D; Goodall A J; Vermote C L; Halford S E
Department of Biochemistry, University of Bristol, U.K.

Biochemistry (UNITED STATES) Dec 4 1990, 29 (48) p10727-33, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The EcoRV restriction/modification system consists of two enzymes that recognize the DNA sequence GATATC. The EcoRV restriction endonuclease cleaves DNA at this site, but the DNA of Escherichia coli **carrying** the EcoRV system is protected from this reaction by the EcoRV methyltransferase. However, in vitro, the EcoRV nuclease also cleaves DNA at most sites that differ from the recognition sequence by one base pair. Though the reaction of the nuclease at these sites is much slower than that at the cognate site, it still appears to be fast enough to cleave the chromosome of the cell into many fragments. The possibility that the EcoRV methyltransferase also protects the noncognate sites on the chromosome was examined. The modification enzyme methylated **alternate** sites in vivo, but these were not the same as the **alternate** sites for the nuclease. The excess methylation was found at GATC sequences, which are also the targets for the dam methyltransferase of E. coli, a protein that is homologous to the EcoRV methyltransferase. Methylation at these sites gave virtually no protection against the EcoRV nuclease: even when the EcoRV methyltransferase had been overproduced, the cellular DNA remained sensitive to the EcoRV nuclease at its noncognate sites. The viability of E. coli **carrying** the EcoRV restriction/modification system was found instead to depend on the activity of DNA ligase. Ligase appears to proofread the EcoRV R/M system in vivo: DNA, cut initially in one strand at a noncognate site for the nuclease, is presumably repaired by ligase before the scission of the second strand.

Tags: Comparative Study; Support, Non-U.S. Gov't
Descriptors: DNA, Bacterial--metabolism--ME; *Deoxyribonucleases, Type II Site-Specific--metabolism--ME; *Escherichia coli--genetics--GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME; Base Sequence; DNA Restriction Enzymes--metabolism--ME; Methylation; **Plasmids** ; Substrate Specificity
CAS Registry No.: 0 (DNA, Bacterial); 0 (Plasmids)
Enzyme No.: EC 2.1.1.- (DNA modification methylase EcoRV); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21 (DNA Restriction Enzymes); EC 3.1.21.- (endodeoxyribonuclease EcoRV); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)
Record Date Created: 19910228
Record Date Completed: 19910228

8/9/59

DIALOG(R)File 155:MEDLINE(R)

08765696 PMID: 2271627

Incorporation of a complete set of deoxyadenosine and thymidine analogues suitable for the study of protein nucleic acid interactions into oligodeoxynucleotides. Application to the EcoRV restriction endonuclease and modification methylase.

Newman P C; Nwosu V U; Williams D M; Cosstick R; Seela F; Connolly B A
Department of Biochemistry (SERC Molecular Recognition Centre), University of Southampton, U.K.

Biochemistry (UNITED STATES) Oct 23 1990, 29 (42) p9891-901, ISSN 0006-2960 Journal Code: 0370623

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

A complete set of dA and T analogues designed for the study of protein DNA interactions has been prepared. These modified bases have been designed by considering the groups on the dA and T bases that are accessible to proteins when these bases are incorporated into double-helical B-DNA [Seeman, N. C., Rosenberg, J. M., & Rich, A. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 804-808]. Each of the positions on the two bases, having the potential to interact with proteins, have been subject to nondisruptive, conservative change. Typically a particular group (e.g., the 6-NH2 of dA or the 5-CH3 of T) has been replaced with a hydrogen atom. Occasionally keto groups (the 2- and 4-keto oxygen atoms of T) have been replaced with sulfur. The base set has been incorporated into the self-complementary dodecamer d(GACGATATCGTC) at the central d(ATAT) sequence. Melting temperature determination shows that the modified bases do not destabilize the double helix. Additionally, circular dichroism spectroscopy shows that almost all the **altered** bases have very little effect on overall oligodeoxynucleotide conformation and that most of the modified oligomers have a B-DNA type structure. d(GATATC) is the recognition sequence for the EcoRV restriction modification system. Initial rate measurements (at a single oligodeoxynucleotide concentration of 20 microM) have been **carried** out with both the EcoRV restriction endonuclease and modification methylase. This has enabled a preliminary identification of the groups of the dA and T bases within the d(GATATC) sequence that make important contacts to both proteins.

Tags: Comparative Study; Support, Non-U.S. Gov't

Descriptors: Adenosine--analogs and derivatives--AA; *DNA-Binding Proteins--metabolism--ME; *Deoxyribonucleases, Type II Site-Specific --metabolism--ME; *Oligodeoxyribonucleotides--chemical synthesis--CS; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME ; *Thymidine--analogs and derivatives--AA; Adenosine--chemical synthesis --CS; Base Sequence; Deoxyadenosines--chemical synthesis--CS; Deoxyadenosines--metabolism--ME; Molecular Conformation; Molecular Sequence Data; Oligodeoxyribonucleotides--metabolism--ME; Protein Binding; Structure-Activity Relationship; Substrate Specificity; Thymidine--chemical synthesis--CS

CAS Registry No.: 0 (DNA-Binding Proteins); 0 (Deoxyadenosines); 0 (Oligodeoxyribonucleotides); 50-89-5 (Thymidine); 58-61-7 (Adenosine)

Enzyme No.: EC 2.1.1.- (DNA modification methylase EcoRV); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (endodeoxyribonuclease EcoRV); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)

Record Date Created: 19910227
Record Date Completed: 19910227

8/9/60

DIALOG(R)File 155:MEDLINE(R)

08659916 PMID: 1976239

Disruption of phase during PCR amplification and cloning of heterozygous target sequences.

Jansen R; Ledley F D

Howard Hughes Medical Institute, Department of Cell Biology and Pediatrics, Baylor College of Medicine, Houston, TX 77030.

Nucleic acids research (ENGLAND) Sep 11 1990, 18 (17) p5153-6, ISSN 0305-1048 Journal Code: 0411011

Contract/Grant No.: HD-24064; HD; NICHD; HD-24186; HD; NICHD

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

PCR amplification of genomic DNA or cDNA has become a standard tool for identification of **mutations** underlying genetic disease. There are inherent limitations in the application of this method in compound heterozygotes. One problem which is encountered is the disruption of phase (linkage) between heterozygous polymorphisms represented on **heterologous** alleles. A test system was used to demonstrate and quantitate the disruption of phase between two polymorphic restriction sites. Phase is disrupted in approximately 1% of the PCR amplified material, possibly due to incomplete chain elongations and subsequent priming on the **heterologous** allele. Phase is disrupted in approximately 1/4 of cloned PCR fragments, possibly due to excision repair of heteroduplexes during cloning. The implications of these disruptions for the use of PCR in identifying **mutations** are discussed.

Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: Gene Amplification; *Heterozygote; *Isomerases--genetics--GE ; *Linkage (Genetics); *Methylmalonyl-CoA Mutase--genetics--GE; *Polymerase Chain Reaction; *Polymorphism, Restriction Fragment Length; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Base Sequence; Cell Line; Cloning, Molecular; Methyltransferases--metabolism--ME; Molecular Sequence Data; Restriction Mapping

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 5. (Isomerases); EC 5.4.99.2 (Methylmalonyl-CoA Mutase)

Record Date Created: 19901024

Record Date Completed: 19901024

8/9/61

DIALOG(R)File 155:MEDLINE(R)

08599201 PMID: 2142667

Probing the function of individual amino acid residues in the DNA binding site of the EcoRI restriction endonuclease by analysing the toxicity of genetically engineered mutants .

Oelgeschlager T; Geiger R; Ruter T; Alves J; Fliess A; Pingoud A

Zentrum Biochemie, Medizinische Hochschule Hannover, F.R.G.

Gene (NETHERLANDS) Apr 30 1990, 89 (1) p19-27, ISSN 0378-1119 Journal Code: 7706761

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

We have developed an assay that allows analysis of the activity of EcoRI restriction endonuclease (ENase) and its **mutants** in vivo. This assay is based on the fact that wild type (wt) EcoRI ENase is toxic for Escherichia coli cells not expressing the EcoRI methyltransferase (MTase). The viability factor defined by the ratio of the viable counts of E. coli cultures having or not having expressed the ecoRIR gene for a defined time is 10(-6) for wt EcoRI ENase and close to one for a totally inactive EcoRI ENase **mutant** . While the EcoRI MTase (M.EcoRI) provides substantial protection against the toxic effects of the wt EcoRI ENase and several of the **mutants** , some **mutants** become more toxic in the presence of M.EcoRI. Twenty-four different DNA-binding-site **mutants** of EcoRI ENase were characterized in their activity in vivo with this assay. The results obtained allow us to conclude that the structural integrity of the region at and around aa 200 seems to be very critical for the enzymatic function of EcoRI ENase: nonconservative replacements there lead to viability factors of 1-10(-2). While our results indicate that the region around aa 144 and 145 is also involved in the EcoRI ENase-catalyzed reaction, it is also evident that the effects of **mutation** there are not as large: viability factors of approx. 10(-3) are obtained even for drastic replacements. These results are discussed in the light of the x-ray structure analysis of an EcoRI ENase-DNA recognition complex.

Tags: Support, Non-U.S. Gov't

8/9/63
DIALOG(R)File 155:MEDLINE(R)

08513379 PMID: 2185235

The DNA adenine methyltransferase **(dam+) gene of bacteriophage T4 reverses the mutator phenotype of an Escherichia coli dam** mutant .

Hall R M
Laboratory for Molecular Biology, CSIRO Division of Biotechnology, North Ryde, NSW, Australia.

Journal of bacteriology (UNITED STATES) May 1990, 172 (5) p2812-3,
ISSN 0021-9193 Journal Code: 2985120R

Descriptors: *Deoxyribonuclease EcoRI--genetics--GE; *Escherichia coli --genetics--GE; Amino Acid Sequence; Bacteriophage lambda--genetics--GE; Binding Sites; DNA, Viral--metabolism--ME; Deoxyribonuclease EcoRI --metabolism--ME; Escherichia coli--enzymology--EN; Escherichia coli --growth and development--GD; Genetic **Vectors** ; Models, Molecular; Molecular Sequence Data; **Mutation** ; **Plasmids** ; Protein Conformation; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME ; Substrate Specificity

CAS Registry No.: 0 (DNA, Viral); 0 (Genetic Vectors); 0 (Plasmids)
Enzyme No.: EC 2.1.1.- (DNA modification methylase EcoRI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (Deoxyribonuclease EcoRI)
Record Date Created: 19900824
Record Date Completed: 19900824

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The mutator phenotype of Escherichia coli dam **mutants** was found to be reversed by introduction of the bacteriophage T4 gene for DNA **adenine methyltransferase** . This precludes a direct role for the E. coli DNA **adenine methyltransferase** in mismatch repair, in addition to its role in strand discrimination, as suggested by earlier studies (S. L. Schlagman, S. Hattman, and M. G. Marinus, J. Bacteriol. 165:896-900, 1986).

Descriptors: Escherichia coli--genetics--GE; *Genes, Structural, Viral; *Methyltransferases--genetics--GE; * **Mutation** ; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); *T-Phages--genetics--GE; Cloning, Molecular; Escherichia coli--enzymology--EN; Phenotype; **Plasmids** ; T-Phages--enzymology--EN

CAS Registry No.: 0 (Plasmids)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19900605
Record Date Completed: 19900605

8/9/64
DIALOG(R)File 155:MEDLINE(R)

08512706 PMID: 2185133

Specificity of Escherichia coli mutD and mutL mutator strains.

Wu T H; Clarke C H; Marinus M G

Department of Pharmacology, University of Massachusetts Medical School, Worcester 01655.

Gene (NETHERLANDS) Mar 1 1990, 87 (1) p1-5, ISSN 0378-1119 Journal Code: 7706761

Contract/Grant No.: GM33233; GM; NIGMS

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The products of the mutD and mutL genes of Escherichia coli are involved in proofreading by DNA polymerase III and DNA adenine MTase (Dam)-dependent mismatch repair, respectively. We have used the **plasmid** -borne bacteriophage P22 mnt gene as a target to determine the types of **mutations** produced in mutL25 and mutD5 strains. Of 60 **mutations** identified from mutL25 cells, 52 were transition **mutations** and of these the AT----GC subset predominated (40 out of 52). The majority of AT----GC **mutations** were found at the same three sites (hotspots). In contrast, transversion **mutations** (47 out of 76) were found about twice as frequently as transitions (28 out of 76) from mutD5 bacteria. Two hotspots were identified but at different sites than those in the mutL25 cells. These results suggest that the proofreading function of DNA polymerase III primarily repairs potential transversion **mutations** while Dam-dependent mismatch repair rectifies potential transition **mutations** .

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: Coliphages--genetics--GE; *Escherichia coli--genetics--GE; *Genes, Bacterial; *Genes, Viral; * **Mutation** ; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); DNA Polymerase III--metabolism --ME; DNA Repair; Escherichia coli--enzymology--EN; Methyltransferases --metabolism--ME; **Plasmids** ; Promoter Regions (Genetics)

CAS Registry No.: 0 (Plasmids)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 2.7.7.- (DNA Polymerase III)

Record Date Created: 19900606
Record Date Completed: 19900606

p85-96, ISSN 0026-8925 Journal Code: 0125036
Contract/Grant No.: GM31839; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

DNA containing the Escherichia coli dam gene and sequences upstream from this gene were cloned from the Clarke-Carbon **plasmids** pLC29-47 and pLC13-42. Promoter activity was localized using pKO expression **vectors** and galactokinase assays to two regions, one 1650-2100 bp and the other beyond 2400 bp upstream of the dam gene. No promoter activity was detected immediately in front of this gene; **plasmid** pDam118, from which the nucleotide sequence of the dam gene was determined, is shown to contain the pBR322 promoter for the primer RNA from the pBR322 rep region present on a 76 bp Sau3A fragment **inserted** upstream of the dam gene in the correct orientation for dam expression. The nucleotide sequence upstream of dam has been determined. An open reading frame (ORF) is present between the nearest promoter region and the dam gene. Codon usage and base frequency analysis indicate that this is expressed as a protein of predicted size 46 kDa. A protein of size close to 46 kDa is expressed from this region, detected using minicell analysis. No function has been determined for this protein, and no significant homology exist between it and sequences in the PIR protein or GenBank DNA databases. This unidentified reading frame (URF) is termed urf-74.3, since it is an URF located at 74.3 min on the E. coli chromosome. Sequence comparisons between the regions upstream of urf-74.3 and the aroB gene show that the aroB gene is located immediately upstream of urf-74.3, and that the promoter activity nearest to dam is found within the aroB structural gene. This activity is relatively weak (about 15% of that of the E. coli gal operon promoter). The promoter activity detected beyond 2400 bp upstream of dam is likely to be that of the aroB gene, and is 3 to 4 times stronger than that found within the aroB gene. Three potential DnaA binding sites, each with homology of 8 of 9 bp, are present, two in the aroB promoter region and one just upstream of the dam gene. Expression through the site adjacent to the dam gene is enhanced 2- to 4-fold in dnaA **mutants** at 38 degrees C. Restriction site comparisons map these regions precisely on the Clarke-Carbon **plasmids** pLC13-42 and pLC29-47, and show that the E. coli ponA (mrcA) gene resides about 6 kb upstream of aroB.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: Bacterial Proteins--genetics--GE; *Escherichia coli --genetics--GE; *Genes, Bacterial; *Methyltransferases--genetics--GE; *Operon; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Alleles; Amino Acid Sequence; Base Sequence; Chromosome Deletion; Chromosome Mapping; Cloning, Molecular; DNA, Bacterial--genetics--GE; Galactokinase--metabolism--ME; Gene Amplification; Molecular Sequence Data; **Mutation** ; **Plasmids** ; Promoter Regions (Genetics); Transcription, Genetic

Molecular Sequence Databank No.: GENBANK/X15162

CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA, Bacterial); 0 (Plasmids)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 2.7.1.6 (Galactokinase)

Record Date Created: 19890925

Record Date Completed: 19890925

8/9/71

DIALOG(R)File 155:MEDLINE(R)

08181294 PMID: 2500421

Autogenous regulation of the Escherichia coli ksgA gene at the level of translation.

van Gemen B; Twisk J; van Knippenberg P H

Department of Biochemistry, Leiden University, The Netherlands.

Journal of bacteriology (UNITED STATES) Jul 1989, 171 (7) p4002-8,

ISSN 0021-9193 Journal Code: 2985120R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Various **plasmids** that contain the Escherichia coli ksgA gene, which encodes a 16S rRNA adenosine dimethyltransferase (methylase), were constructed. In one of these **plasmids** , the DNA encoding the N-terminal part of the methylase was fused to the lacZ gene, and in another construct, the ksgA gene contained a deletion which resulted in a truncated version of the methylase. When a cell contained one **plasmid** directing the synthesis of the intact, active methylase and another **plasmid** encoding the methylase-beta-galactosidase protein, production of the latter product became strongly **reduced** . Likewise, synthesis of the truncated version of the methylase was diminished when the cell at the same time contained a **plasmid** producing the complete enzyme. These results were partly substantiated by in vitro experiments with a coupled transcription-translation assay system. By using a recently developed gel electrophoresis system for measuring protein-nucleic acid interactions, a specific binding of the ksgA methylase with its own mRNA could be established. Our results demonstrate that the expression of the ksgA gene can be, at least partly, autogenously controlled at the level of translation.

Descriptors: Escherichia coli--genetics--GE; *Genes, Bacterial; *RNA, Ribosomal--genetics--GE; *RNA, Ribosomal, 16S--genetics--GE; *Regulatory Sequences, Nucleic Acid; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; *Translation, Genetic; Escherichia coli --enzymology--EN; Lac Operon; **Plasmids** ; RNA, Messenger--metabolism--ME; Recombinant Fusion Proteins--biosynthesis--BI; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--biosynthesis--BI; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME; Suppression, Genetic; Transcription, Genetic; beta-Galactosidase--genetics--GE

CAS Registry No.: 0 (Plasmids); 0 (RNA, Messenger); 0 (RNA, Ribosomal); 0 (RNA, Ribosomal, 16S); 0 (Recombinant Fusion Proteins)

Enzyme No.: EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.2.1.23 (beta-Galactosidase)

Record Date Created: 19890803
Record Date Completed: 19890803

8/9/72
DIALOG(R)File 155:MEDLINE(R)

08142982 PMID: 3074016
The SalI (SalGI) restriction-modification system of Streptomyces albus G.
Rodicio M R; Chater K F
Departamento de Microbiologia, Universidad de Oviedo, Spain.
Gene (NETHERLANDS) Dec 25 1988, 74 (1) p39-42, ISSN 0378-1119 Journal Code: 7706761
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The salIR and salM genes of Streptomyces albus G specify the SalGI (SalI) restriction enzyme and its cognate methyltransferase, respectively. These enzymes are responsible for restriction and modification of bacteriophages. Some phages **carry** genes that interfere with SalI-specific modification. The sal genes have been cloned in a Streptomyces host- **vector** system. Use of the cloned DNA as a hybridization probe reveals that sal **mutants** frequently arise from transposition of a DNA segment of approx. 1 kb into the sal genes. Some, but not all, other bacteria that produce SalGI isoschizomers contain nucleotide sequences that hybridize with sal DNA. (13 Refs.)

Descriptors: *Bacterial Proteins--metabolism--ME; *Deoxyribonucleases,

Type II Site-Specific--metabolism--ME; *Streptomyces--enzymology--EN; Bacterial Proteins--genetics--GE; DNA, Bacterial--metabolism--ME; DNA, Viral--metabolism--ME; Deoxyribonucleases, Type II Site-Specific--genetics --GE; Genes, Bacterial; Recombinant Proteins--genetics--GE; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME; Streptomyces --genetics--GE

CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA, Bacterial); 0 (DNA, Viral); 0 (Recombinant Proteins)

Enzyme No.: EC 2.1.1.- (DNA modification methylase SalGI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (endodeoxyribonuclease SalI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)

Record Date Created: 19890626

Record Date Completed: 19890626

8/9/73

DIALOG(R)File 155:MEDLINE(R)

08102613 PMID: 2854098

A simple and rapid method to obtain substitution mutations **in Escherichia coli: isolation of a dam deletion/** insertion mutation .

Parker B; Marinus M G

Department of Pharmacology, University of Massachusetts Medical School, Worcester 01655.

Gene (NETHERLANDS) Dec 20 1988, 73 (2) p531-5, ISSN 0378-1119 Journal Code: 7706761

Contract/Grant No.: GM30330; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

We describe the isolation of a strain of Escherichia coli bearing a deletion/ **insertion** (i.e., a substitution **mutation**) in the dam gene (dam-16). The **mutagenesis** protocol used should be applicable to any cloned non-essential gene of E. coli. The substitution **mutation** confers resistance to kanamycin and can easily be transferred to other strains by standard genetic techniques. The amount of Dam methyltransferase (MTase) in dam-16 strains as determined either in vitro or in vivo is below the level of detection. We conclude that the Dam MTase is not required for viability of E. coli.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: Chromosome Deletion; *DNA Transposable Elements; *Escherichia coli--genetics--GE; *Genes, Bacterial; *Genes, Structural; * **Mutation** ; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific) --genetics--GE; Chromosome Mapping; Chromosomes, Bacterial; Crosses, Genetic; Escherichia coli--enzymology--EN; Genotype; Transduction, Genetic

CAS Registry No.: 0 (DNA Transposable Elements)

Enzyme No.: EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19890605

Record Date Completed: 19890605

8/9/74

DIALOG(R)File 155:MEDLINE(R)

08091911 PMID: 3071738

Increased kasugamycin sensitivity in Escherichia coli caused by the presence of an inducible erythromycin resistance (erm) gene of Streptococcus pyogenes.

Suvorov A N; van Gemen B; van Knippenberg P H

Department of Biochemistry, Leiden University, The Netherlands.

Molecular & general genetics - MGG (GERMANY, WEST) Dec 1988, 215 (1) p152-5, ISSN 0026-8925 Journal Code: 0125036
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

An inducible erythromycin resistance gene (erm) of Streptococcus pyogenes was introduced into Escherichia coli by **transformation** with a **plasmid** . The recipient E. coli cells were either kasugamycin sensitive (wildtype) or kasugamycin resistant (ksgA). The MIC values of erythromycin increased from 150 micrograms/ml to greater than 3000 micrograms/ml for E. coli. An extract of **transformed** cells, particularly a high-salt ribosomal wash, contained an enzyme that was able to methylate 23S rRNA from untransformed cells in vitro; however, 23S rRNA from **transformed** cells was not a substrate for methylation by such an extract. 165 rRNA and 30S ribosomal subunits of either the wild type or a kasugamycin resistant (ksgA) **mutant** were not methylated in vitro. **Transformation** of E. coli by the erm-containing **plasmid** led to a **reduction** of the MIC values for kasugamycin. This happened in wild-type as well as in ksgA cells. However, in vitro experiments with purified ksgA encoded methylase demonstrated that also in erm **transformed** E. coli, the ksgA encoded enzyme was active in wild-type, but not in ksgA cells. It was also shown by in vitro experiments that ribosomes from erm ksgA cells have become sensitive to kasugamycin. Our experiments show that in vivo methylation of 23S rRNA, presumably of the adenosine at position 2058, leads to enhanced resistance to erythromycin and to **reduced** resistance to kasugamycin. This, together with previous data, argues for a close proximity of the two sites on the ribosome that are substrates for adenosine dimethylation.

Descriptors: *Aminoglycosides; *Drug Resistance, Microbial--genetics--GE; *Escherichia coli--genetics--GE; *Genes, Bacterial; *Streptococcus pyogenes --genetics--GE; Anti-Bacterial Agents--pharmacology--PD; Erythromycin --pharmacology--PD; Escherichia coli--drug effects--DE; Escherichia coli --enzymology--EN; Methyltransferases--genetics--GE; R Factors; Ribosomes --drug effects--DE; Streptococcus pyogenes--drug effects--DE; **Transformation** , Genetic

CAS Registry No.: 0 (Aminoglycosides); 0 (Anti-Bacterial Agents); 0 (R Factors); 114-07-8 (Erythromycin); 6980-18-3 (kasugamycin)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.66 (rRNA (**adenosine** -O-2'-) **methyltransferase**)

Record Date Created: 19890519
Record Date Completed: 19890519

8/9/75
DIALOG(R)File 155:MEDLINE(R)

08069502 PMID: 2648008

Base methylation and local DNA helix stability. Effect on the kinetics of cruciform extrusion.

Murchie A I; Lilley D M
Department of Biochemistry, The University, Dundee, U.K.

Journal of molecular biology (ENGLAND) Feb 5 1989, 205 (3) p593-602, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

We have studied the effect of base methylation on the rate of cruciform extrusion. A number of inverted repeats with central restriction sites were methylated at N6-adenine and C-5-cytosine, and rate constants for cruciform extrusion at 37 degrees C were measured. The effect of A-methylation at two bases was to enhance the rate for extrusion by nearly fourfold, while C-methylation lead to **reduced** extrusion rates, by factors of 1.7 and 2.7. The bkb inverted repeat, which has a central GGATCC sequence, was

independently and simultaneously methylated at adenine and cytosine. It was found that the effects of the two kinds of modification could be treated effectively independently. The results reveal the local helical destabilization and stabilization due to A and C-methylation, respectively.

Tags: Support, Non-U.S. Gov't

Descriptors: *DNA, Superhelical--metabolism--ME; Base Composition; Base Sequence; Escherichia coli; Kinetics; Methylation; Molecular Sequence Data; **Plasmids** ; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific) --metabolism--ME

CAS Registry No.: 0 (DNA, Superhelical); 0 (Plasmids)

Enzyme No.: EC 2.1.1.- (DNA modification methylase EcoRI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19890505

Record Date Completed: 19890505

8/9/76

07925688 PMID: 3053648

Molecular cloning, sequencing, and mapping of the bacteriophage T2 dam gene.

Miner Z; Hattman S

Department of Biology, University of Rochester, New York 14627.

Journal of bacteriology (UNITED STATES) Nov 1988, 170 (11) p5177-84, ISSN 0021-9193 Journal Code: 2985120R

Contract/Grant No.: GM29227; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Bacteriophage T2 codes for a DNA-(**adenine** -N6) **methyltransferase** (Dam), which is able to methylate both cytosine- and hydroxymethylcytosine-containing DNAs to a greater extent than the corresponding methyltransferase encoded by bacteriophage T4. We have cloned and sequenced the T2 dam gene and compared it with the T4 dam gene. In the Dam coding region, there are 22 nucleotide differences, 4 of which result in three coding differences (2 are in the same codon). Two of the amino acid **alterations** are located in a region of homology that is shared by T2 and T4 Dam, Escherichia coli Dam, and the modification enzyme of Streptococcus pneumoniae, all of which methylate the sequence 5' GATC 3'. The T2 dam and T4 dam promoters are not identical and appear to have slightly different efficiencies; when fused to the E. coli lacZ gene, the T4 promoter produces about twofold more beta-galactosidase activity than does the T2 promoter. In our first attempt to isolate T2 dam, a truncated gene was cloned on a 1.67-kilobase XbaI fragment. This construct produces a chimeric protein composed of the first 163 amino acids of T2 Dam followed by 83 amino acids coded by the pUC18 **vector** . Surprisingly, the chimera has Dam activity, but only on cytosine-containing DNA. Genetic and physical analyses place the T2 dam gene at the same respective map location as the T4 dam gene. However, relative to T4, T2 contains an **insertion** of 536 base pairs 5' to the dam gene. Southern blot hybridization and computer analysis failed to reveal any homology between this **insert** and either T4 or E. coli DNA.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: Cloning, Molecular; *Escherichia coli--genetics--GE; *Genes, Structural; *Genes, Viral; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; *T-Phages--genetics--GE; Amino Acid Sequence; Base Sequence; Blotting, Southern; Molecular Sequence Data; Nucleic Acid Hybridization; **Plasmids** ; Restriction Mapping; T-Phages--enzymology--EN

Molecular Sequence Databank No.: GENBANK/M22342

CAS Registry No.: 0 (Plasmids)

Enzyme No.: EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19881206

mutation within a dam recognition sequence adjacent to the required 19 base pairs of the inside end did not **reduce** the magnitude of dam regulation.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *DNA Transposable Elements; Bacterial Proteins--physiology --PH; Base Sequence; DNA, Recombinant; Escherichia coli--enzymology--EN; Escherichia coli--genetics--GE; Methyltransferases--physiology--PH; **Mutation** ; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)

CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA Transposable Elements); 0 (DNA, Recombinant)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19880512

Record Date Completed: 19880512

8/9/80

DIALOG(R)File 155:MEDLINE(R)

07561119 PMID: 2820843

Formation of MboII vectors **and cassettes using asymmetric MboII linkers.**

Gayle R B; Auger E A; Gough G R; Gilham P T; Bennett G N

Department of Biochemistry, Rice University, Houston, TX 77251.

Gene (NETHERLANDS) 1987, 54 (2-3) p221-8, ISSN 0378-1119 Journal Code: 7706761

Contract/Grant No.: GM07833; GM; NIGMS; GM26437; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Class-IIS restriction endonucleases such as MboII cleave DNA at a specified distance away from their recognition sequences. This feature was exploited to cleave DNA at previously inaccessible locations by preparing special asymmetric linker/adapters containing the MboII recognition sequence. These could be joined to DNA fragments and subsequently cleaved by MboII. Attachment of a 3' phosphate to one of the two different oligodeoxynucleotides comprising the asymmetric duplex prevented ligation at the improper end of the linker. **Plasmids** were constructed containing a unique BamHI or BclI site between the recognition and cleavage site of MboII. These sites were used to introduce a **foreign** fragment into the **plasmid** at a position permitting MboII to cleave within the newly **inserted** fragment. Once cleaved at the unique MboII site, another DNA fragment was **inserted** . DNA was thus **inserted** at a sequence not previously accessible to specific cleavage by a restriction enzyme. A cassette containing an identifiable marker, the lac operator, between two oppositely oriented MboII/BamHI linkers was made and tested in a random **insertion** linker **mutagenesis** experiment.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.

Descriptors: DNA Restriction Enzymes--metabolism--ME; *Deoxyribonucleases , Type II Site-Specific; *Genetic **Vectors** ; *Methyltransferases --metabolism--ME; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Base Sequence; Escherichia coli--genetics--GE; **Plasmids** ; Substrate Specificity

CAS Registry No.: 0 (Genetic Vectors); 0 (Plasmids)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (DNA modification methylase MboII); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21 (DNA Restriction Enzymes); EC 3.1.21.- (endodeoxyribonuclease MboII); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)

Record Date Created: 19871119

Record Date Completed: 19871119

8/9/85
DIALOG(R)File 155:MEDLINE(R)

07050281 PMID: 3512529

Direct role of the Escherichia coli Dam DNA methyltransferase in methylation-directed mismatch repair.

Schlagman S L; Hattman S; Marinus M G

Journal of bacteriology (UNITED STATES) Mar 1986, 165 (3) p896-900, ISSN 0021-9193 Journal Code: 2985120R
Contract/Grant No.: GM29227; GM; NIGMS; GM30330; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The T4 dam+ gene has been cloned (S. L. Schlagman and S. Hattman, Gene 22:139-156, 1983) and transferred into an Escherichia coli dam-host. In this host, the T4 Dam- DNA methyltransferase methylates mainly, if not exclusively, the sequence 5'-GATC-3'; this sequence specificity is the same as that of the E. coli Dam enzyme. Expression of the cloned T4 dam+ gene suppresses almost all the phenotypic traits associated with E. coli dam

mutants , with the exception of hypermutability. In wild-type hosts, 20- to 500-fold overproduction of the E. coli Dam methylase by **plasmids** containing the cloned E. coli dam+ gene results in a hypermutability phenotype (G.E. Herman and P. Modrich, J. Bacteriol. 145:644-646, 1981; M.G. Marinus, A. Poteete, and J.A. Arraj, Gene 28:123-125, 1984). In contrast, the same high level of T4 Dam methylase activity, produced by **plasmids** containing the cloned T4 dam+ gene, does not result in hypermutability. To account for these results we propose that the E. coli Dam methylase may be directly involved in the process of methylation-instructed mismatch repair and that the T4 Dam methylase is unable to substitute for the E. coli enzyme.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: *DNA Repair; *DNA, Bacterial--metabolism--ME; *Escherichia coli--enzymology--EN; *Methyltransferases--metabolism--ME; Cloning, Molecular; Escherichia coli--genetics--GE; Methylation; Methyltransferases --genetics--GE; **Mutation** ; Phenotype; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Substrate Specificity; T-Phages--enzymology--EN; T-Phages--genetics--GE

CAS Registry No.: 0 (DNA, Bacterial)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19860409

Record Date Completed: 19860409

8/9/86

DIALOG(R)File 155:MEDLINE(R)

06968320 PMID: 3905517

Nucleotide sequence of the ksgA gene of Escherichia coli: comparison of methyltransferases **effecting dimethylation of** adenosine **in ribosomal RNA.**

van Buul C P; van Knippenberg P H

Gene (NETHERLANDS) 1985, 38 (1-3) p65-72, ISSN 0378-1119 Journal Code: 7706761

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The ksgA gene of Escherichia coli encodes a methyltransferase (MeT) that specifically dimethylates two adjacent adenosines near the 3' end of 16S RNA in the 30S particle. Its inactivation leads to kasugamycin (Ksg) resistance. Several **plasmids** were constructed with **inserts** which complemented chromosomal ksgA **mutations** . One of these **inserts** was sequenced and found to contain an open reading frame (ORF) sufficient to code for the previously identified 30-kDal MeT. We have compared the amino acid (aa) sequence of the ksgA-encoded enzyme with three published sequences of MeT involved in dimethylation of an adenosine residue in 23S RNA and rendering the organisms resistant to the MLS antibiotics. The homologous patches in the sequences of all four enzymes suggest that those might correspond to contact points for the common substrates, e.g., for the adenosine residue(s) and S-adenosylmethionine (SAM).

Tags: Comparative Study

Descriptors: *Aminoglycosides; *Anti-Bacterial Agents; *Escherichia coli --genetics--GE; *Genes, Bacterial; *Methyltransferases--genetics--GE; *RNA, Ribosomal--metabolism--ME; Amino Acid Sequence; Base Sequence; Cloning, Molecular; Codon; Drug Resistance, Microbial; Gene Expression Regulation; Methylation; Methyltransferases--metabolism--ME

Molecular Sequence Databank No.: GENBANK/M11054

CAS Registry No.: 0 (Aminoglycosides); 0 (Anti-Bacterial Agents); 0 (Codon); 0 (RNA, Ribosomal); 6980-18-3 (kasugamycin)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.48 (rRNA(**adenine** -N6)- **methyltransferase**)

Record Date Created: 19860121

Record Date Completed: 19860121

8/9/87

06807038 PMID: 6442874

Inducible erythromycin resistance in bacteria.

Weisblum B

British medical bulletin (ENGLAND) Jan 1984, 40 (1) p47-53, ISSN 0007-1420 Journal Code: 0376542

Contract/Grant No.: AI-18283; AI; NIAID

Document type: Journal Article; Review

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

(59 Refs.)

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.

Descriptors: *Bacteria--drug effects--DE; *Erythromycin--pharmacology--PD ; Bacteria--genetics--GE; Bacterial Proteins--biosynthesis--BI; Base Sequence; Drug Resistance, Microbial; Genes, Bacterial; Genes, Regulator; Lincomycin--pharmacology--PD; Methylation; Methyltransferases--biosynthesis --BI; Models, Genetic; **Mutation** ; **Plasmids** ; RNA, Ribosomal--metabolism --ME; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Translation, Genetic; Virginiamycin--pharmacology--PD

CAS Registry No.: 0 (Bacterial Proteins); 0 (Plasmids); 0 (RNA, Ribosomal); 11006-76-1 (Virginiamycin); 114-07-8 (Erythromycin); 154-21-2 (Lincomycin)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19850711

Record Date Completed: 19850711

8/9/88

06794722 PMID: 3887134

Expression of the Escherichia coli dam methylase in Saccharomyces cerevisiae: effect of in vivo adenine methylation on genetic recombination and mutation .

Hoekstra M F; Malone R E

Molecular and cellular biology (UNITED STATES) Apr 1985, 5 (4) p610-8, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: R01-GM 29172; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The Escherichia coli DNA adenine methylase (dam) gene has been introduced into Saccharomyces cerevisiae on a yeast-E. coli shuttle **vector** . Sau3AI, MboI, and DpnI restriction enzyme digests and Southern hybridization analysis indicated that the dam gene is expressed in yeast cells and methylates GATC sequences. Analysis of digests of total genomic DNA indicated that some GATC sites are not sensitive to methylation. The failure to methylate may reflect an inaccessibility to the methylase due to chromosome structure. The effects of this in vivo methylation on the processes of recombination and **mutation** in mitotic cells were determined. A small but definite general increase was found in the frequency of mitotic recombination. A similar increase was observed for reversion of some auxotrophic markers; other markers demonstrated a small decrease in **mutation** frequency. The effects on **mutation** appear to be locus (or allele) specific. Recombination in meiotic cells was measured and was not

detectably **altered** by the presence of 6-methyladenine in GATC sequences.
Tags: Support, U.S. Gov't, P.H.S.
Descriptors: DNA, Fungal--genetics--GE; *Methylation; *Methyltransferases --genetics--GE; * **Mutation** ; *Recombination, Genetic; *Saccharomyces cerevisiae--genetics--GE; Base Sequence; DNA Repair; Escherichia coli --enzymology--EN; Escherichia coli--genetics--GE; Meiosis; Mitosis; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)
CAS Registry No.: 0 (DNA, Fungal)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19850613
Record Date Completed: 19850613

8/9/89
DIALOG(R)File 155:MEDLINE(R)

06689053 PMID: 3880739
Extent of equilibrium perturbation of the DNA helix upon enzymatic methylation of adenine residues.
Cheng S C; Herman G; Modrich P
Journal of biological chemistry (UNITED STATES) Jan 10 1985, 260 (1) p191-4, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: GM23719; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The extent of equilibrium perturbation of the DNA helix associated with enzymatic methylation of dA residues has been determined by the agarose gel electrophoresis band-shift method. Utilization of EcoRI methylase under conditions of **reduced** specificity together with Escherichia coli dam methylase permitted modification of up to 300 dA residues/ **plasmid** pBR322 dimer. A conformational change associated with methylation was observed, with the magnitude of the transition being linear with extent of modification of relaxed DNA circles. The conformational change corresponds to an unwinding of the DNA helix by 0.5 degrees/methyl group transferred to relaxed molecules. The magnitude of the effect was independent of temperature from 5-37 degrees C indicating that it is not the consequence of a thermal transition within this range.
Tags: Support, U.S. Gov't, P.H.S.
Descriptors: **Adenine** ; *DNA; * **Methyltransferases** --metabolism--ME; *Nucleic Acid Conformation; * **Plasmids** ; Adenine--analogs and derivatives --AA; Escherichia coli--enzymology--EN; Kinetics; Methylation; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Thermodynamics
CAS Registry No.: 0 (Plasmids); 443-72-1 (6-methyladenine); 73-24-5 (Adenine); 9007-49-2 (DNA)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (DNA modification methylase EcoRI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19850215
Record Date Completed: 19850215

8/9/90
DIALOG(R)File 155:MEDLINE(R)

06639185 PMID: 6092220
S1 nuclease mapping of the phage Mu mom gene promoter: a model for the regulation of mom expression.
Hattman S; Ives J
Gene (NETHERLANDS) Jul-Aug 1984, 29 (1-2) p185-98, ISSN 0378-1119 Journal Code: 7706761
Contract/Grant No.: GM-29227; GM; NIGMS

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The mom gene of bacteriophage Mu encodes a DNA modification function. Expression of this modification requires the host Escherichia coli Dam (DNA-adenine methylase) function and the transacting phage Mu Dad function. The mom gene was subcloned into a variety of sites on **plasmid** pBR322. **Insertions** were made into the HincII and PvuI sites within the amp gene and into the ClaI site of the tet gene promoter. The only clones found were those in which the orientation of the mom gene prevents its transcription from the **vector** promoter(s), suggesting that constitutive expression of mom from a **foreign** promoter can occur independently of Dad function but is lethal for the cell. Employing S1 nuclease mapping, we have identified two Mu mRNA transcripts: (1) the gin transcript extends into the gin-mon intercistronic divide and terminates downstream from the BclI site; and (2) the mom transcript appears to initiate about 74 bp upstream from the BclI site, 12 bp downstream from a promoter-like sequence. Production of the mom transcript is dependent on the host Dam activity and on Dad transactivation. In contrast, the gin transcript is produced independently of Dam and Dad functions; the gin transcript may extend into the mom gene, but it appears to be either degraded at the 3' end or differentially terminated. We propose that regulation of mom gene transcription involves both positive and **negative** regulatory proteins, and that binding of the Dad protein (a "late" Mu protein) is required for transcription initiation by the host RNA polymerase. However, Dad protein action may be inhibited by prior binding of a repressor to the mom operator, located farther upstream. We propose that this repressor (encoded by a phage or host gene) binds to the operator only when there is no active Dam enzyme present, i.e., when there is no methylation of (or methylase binding to) the G-A-T-C sites within the mom operator.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: *Coliphages--genetics--GE; *Escherichia coli--genetics--GE; *Genes, Structural; *Genes, Viral; *Methyltransferases--genetics--GE; *Operon; Aspergillus Nuclease S1; Base Sequence; Cloning, Molecular; DNA Restriction Enzymes; Endonucleases; **Plasmids** ; Site-Specific DNA-**Methyltransferase** (**Adenine** -Specific); Transcription, Genetic; Translation, Genetic

CAS Registry No.: 0 (Plasmids)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.- (Endonucleases); EC 3.1.21 (DNA Restriction Enzymes); EC 3.1.30.1 (Aspergillus Nuclease S1)

Record Date Created: 19841213
Record Date Completed: 19841213

8/9/91
DIALOG(R)File 155:MEDLINE(R)

06546843 PMID: 6376282

Correlation of DNA adenine methylase activity with spontaneous mutability in Escherichia coli K-12.

Marinus M G; Poteete A; Arraj J A

Gene (NETHERLANDS) Apr 1984, 28 (1) p123-5, ISSN 0378-1119 Journal Code: 7706761

Contract/Grant No.: GM30330; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Using a multicopy **plasmid** in which the tac promoter has been placed in front of the dam gene of Escherichia coli K-12, we show that levels of DNA adenine methylase activity are correlated with the spontaneous **mutation**

frequency.
Tags: Support, U.S. Gov't, P.H.S.
Descriptors: Bacterial Proteins--genetics--GE; *Escherichia coli --genetics--GE; *Methyltransferases--genetics--GE; * **Mutation** ; Bacterial Proteins--physiology--PH; DNA Repair; DNA, Bacterial--metabolism--ME; Methyltransferases--physiology--PH; **Plasmids** ; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)
CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA, Bacterial); 0 (Plasmids)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19840727
Record Date Completed: 19840727

8/9/92
DIALOG(R)File 155:MEDLINE(R)

06524576 PMID: 6327647
Transfer of recombinant plasmids **containing the gene for DpnII DNA methylase into strains of Streptococcus pneumoniae that produce DpnI or DpnII restriction endonucleases.**
Lacks S A; Springhorn S S
Journal of bacteriology (UNITED STATES) Jun 1984, 158 (3) p905-9, ISSN 0021-9193 Journal Code: 2985120R
Contract/Grant No.: GMAI29721; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Plasmid transfer via the **transformation** pathway of Streptococcus pneumoniae was weakly restricted by the DpnI or DpnII restriction endonuclease, either of which gave a **reduction** only to 0.4, compared with phage infection, which was restricted to 10(-5). The greater sensitivity of **plasmid** transfer compared with chromosomal **transformation** , which was not at all restricted, can be attributed to partially double-stranded intermediates formed from two complementary donor fragments. However, clustering of potential restriction sites in the **plasmids** increased the probability of escape from restriction. The recombinant **plasmid** pMP10 , in which the gene for the DpnII DNA methylase was cloned, can be transferred to strains that contain neither restriction enzyme or that contain DpnII as readily as can the **vector** pMP5 . Introduction of pMP10 raised the level of methylase by five times the level normally present in DpnII strains. Transfer of pMP10 to DpnI -containing strains was infrequent, presumably owing to the suicidal methylation of DNA which rendered it susceptible to the host endonuclease. The few clones in which pMP10 was established had lost DpnI . **Loss** of the **plasmid** after curing of the cell eliminated the methylase but did not restore DpnI . Although this **loss** of DpnI could result from spontaneous **mutation** , its relatively high frequency, 0.1% suggested that the **loss** was due to a regulatory shift.
Tags: Support, U.S. Gov't, P.H.S.
Descriptors: DNA Restriction Enzymes--genetics--GE; *DNA, Recombinant --metabolism--ME; *Deoxyribonucleases, Type II Site-Specific; *Genes, Bacterial; *Genes, Structural; *Methyltransferases--genetics--GE; * **Plasmids** ; *Streptococcus pneumoniae--genetics--GE; Base Sequence; Chromosomes, Bacterial--physiology--PH; Cloning, Molecular; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Streptococcus pneumoniae --enzymology--EN
CAS Registry No.: 0 (DNA, Recombinant); 0 (Plasmids)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21 (DNA Restriction Enzymes); EC 3.1.21.- (endodeoxyribonuclease DpnI); EC 3.1.21.- (endodeoxyribonuclease MboI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)

Record Date Created: 19840713
Record Date Completed: 19840713

8/9/93
DIALOG(R)File 155:MEDLINE(R)

06306572 PMID: 6316110
Insertion mutations **in the dam gene of Escherichia coli K-12.**
Marinus M G; Carraway M; Frey A Z; Brown L; Arraj J A
Molecular & general genetics - MGG (GERMANY, WEST) 1983, 192 (1-2) p288-9, ISSN 0026-8925 Journal Code: 0125036
Contract/Grant No.: GM30330; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The dam gene of E. coli can be inactivated by **insertion** of Tn9 or Mud phage. Strains bearing these **mutations** are viable indicating that the dam gene product is dispensable.
Tags: Comparative Study; Support, U.S. Gov't, P.H.S.
Descriptors: *Escherichia coli--genetics--GE; *Genes, Bacterial; *Methyltransferases--genetics--GE; DNA Transposable Elements; **Mutation** ; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)
CAS Registry No.: 0 (DNA Transposable Elements)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19840127
Record Date Completed: 19840127

8/9/94
DIALOG(R)File 155:MEDLINE(R)

06213951 PMID: 6309166
Effects of 5'deoxy-5'-methylthioadenosine on the metabolism of S-adenosyl methionine.
Dante R; Arnaud M; Niveleau A
Biochemical and biophysical research communications (UNITED STATES) Jul 18 1983, 114 (1) p214-21, ISSN 0006-291X Journal Code: 0372516
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The treatment of **transformed** rat cells with micromolar amounts of 5'deoxy 5'methyl thioadenosine induces rapid effects on the rate of methylation of DNA concomitantly with **alterations** of intracellular pools of S-adenosyl methionine and S-adenosyl homocysteine. Pulse chase labelling experiments indicate that 5'deoxy 5'methylthioadenosine does not inhibit the degradation of S-adenosyl homocysteine but inhibits the consumption of S-adenosyl methionine. In vitro transmethylation assays performed with **heterologous** DNA show that low doses of the thioethernucleoside do not significantly affect the DNA methyltransferase activity of cellular extracts. The biological role of 5'deoxy 5'methylthioadenosine, a natural molecule formed during the synthesis of polyamines is discussed.
Tags: Support, Non-U.S. Gov't
Descriptors: *Adenosine--analogs and derivatives--AA; *Deoxyadenosines; *S-Adenosylmethionine--metabolism--ME; *Thionucleosides--pharmacology--PD; Adenosine--pharmacology--PD; Animals; Cell Line; Cell **Transformation** , Neoplastic; DNA (Cytosine-5-)-Methyltransferase--metabolism--ME; Kidney --metabolism--ME; Kinetics; Rats; Sarcoma Viruses, Avian--genetics--GE; Sulfur Radioisotopes--diagnostic use--DU
CAS Registry No.: 0 (Deoxyadenosines); 0 (Sulfur Radioisotopes); 0

(Thionucleosides); 2457-80-9 (5'-methylthioadenosine); 29908-03-0 (S-Adenosylmethionine); 58-61-7 (Adenosine)
Enzyme No.: EC 2.1.1.37 (DNA (Cytosine-5-)-Methyltransferase)
Record Date Created: 19830909
Record Date Completed: 19830909

8/9/95
DIALOG(R)File 155:MEDLINE(R)

06145169 PMID: 6406429
A complex attenuator **regulates inducible resistance to macrolides, lincosamides, and streptogramin type B antibiotics in Streptococcus sanguis.**
Horinouchi S; Byeon W H; Weisblum B
Journal of bacteriology (UNITED STATES) Jun 1983, 154 (3) p1252-62, ISSN 0021-9193 Journal Code: 2985120R
Contract/Grant No.: AI-18283; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Macrolide-lincosamide-streptogramin B resistance specified by Streptococcus sanguis **plasmid** pAM77 involves an adenine methylase, whose synthesis, demonstrable both phenotypically and by analysis of methionine-labeled proteins made in Bacillus subtilis minicells, is inducible by erythromycin, lincomycin, and streptogramin type B antibiotics. Localization of the methylase structural gene, including its control region in DNA fragments obtained with restriction endonucleases, has been deduced from DNA blot experiments with characterized target and probe DNAs from other streptococci, combined with DNA sequence analysis and comparison of the putative streptococcal methylase sequence with that of a cognate methylase in staphylococcal **plasmid** pE194. The streptococcal methylase migrates electrophoretically in polyacrylamide gels with the mobility of a 29,000-dalton protein. The sequence organization of the putative streptococcal methylase mRNA leader sequence partially resembles its staphylococcal counterpart and can support a similar mechanism of secondary structure rearrangement leading to methylase synthesis. The deduced 5' leader sequence preceding the pAM77 methylase structural gene sequence comprises approximately 155 nucleotides within which one can identify a putative control peptide 36 amino acid residues in length (in contrast to 19 in the pE194 peptide) and at least 14 possible classes of overlapping inverted complementary repeat sequences (in contrast to 3 in the pE194 control region), one of which can sequester the sequence AGGAG 7 nucleotides upstream from the putative (methionine) start codon of the streptococcal methylase. Comparison of the pAM77 and pE194 methylase amino acid sequences and their respective nucleotide sequences shows 51% conservation of amino acid residues (124 of 244) and 59% conservation of nucleotide residues (433 of 738), which suggests a common origin for the two methylase structural gene sequences. Differences in mRNA base composition associated with conserved amino acid residues occur mostly in the third nucleotide ("wobble") position of codons and may reflect adaptation of methylase genes to optimal expression in host cells with differing codon use patterns.
Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.
Descriptors: *DNA (Cytosine-5-)-Methyltransferase--genetics--GE; *Genes, Structural; *Macrolides; *Methyltransferases--genetics--GE; *Operon; *R Factors; *Streptococcus sanguis--genetics--GE; Amino Acid Sequence; Anti-Bacterial Agents--pharmacology--PD; Base Sequence; Codon; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Streptococcus sanguis--drug effects--DE; Streptococcus sanguis--enzymology--EN; Virginiamycin--pharmacology--PD
Molecular Sequence Databank No.: GENBANK/K00551
CAS Registry No.: 0 (Anti-Bacterial Agents); 0 (Codon); 0

inactivity of the main promoter of the salI operon. In contrast to salIR, salIM was functional in E. coli. Preliminary S1 nuclease mapping experiments suggest that the **alternative** promoter of the MTase gene can initiate transcription in the **heterologous** , as well as in the homologous host.

Tags: Support, Non-U.S. Gov't

Descriptors: Deoxyribonucleases, Type II Site-Specific--biosynthesis--BI; *Recombinant Proteins--biosynthesis--BI; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--biosynthesis--BI; *Streptomyces--enzymology--EN ; Cloning, Molecular--methods--MT; Deoxyribonucleases, Type II Site-Specific--genetics--GE; Escherichia coli--metabolism--ME; Gene Expression; Genes, Bacterial; Operon; Promoter Regions (Genetics); Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Streptomyces--genetics--GE

CAS Registry No.: 0 (Recombinant Proteins)

Enzyme No.: EC 2.1.1.- (DNA modification methylase SalGI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (endodeoxyribonuclease SalI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)

Gene Symbol: salIM; salIR

Record Date Created: 19950811

Record Date Completed: 19950811

11/9/3

08659916 PMID: 1976239

Disruption of phase during PCR amplification and cloning of heterozygous target sequences.

Jansen R; Ledley F D

Howard Hughes Medical Institute, Department of Cell Biology and Pediatrics, Baylor College of Medicine, Houston, TX 77030.

Nucleic acids research (ENGLAND) Sep 11 1990, 18 (17) p5153-6, ISSN 0305-1048 Journal Code: 0411011

Contract/Grant No.: HD-24064; HD; NICHD; HD-24186; HD; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

PCR amplification of genomic DNA or cDNA has become a standard tool for identification of **mutations** underlying genetic disease. There are inherent limitations in the application of this method in compound heterozygotes. One problem which is encountered is the disruption of phase (linkage) between heterozygous polymorphisms represented on **heterologous** alleles. A test system was used to demonstrate and quantitate the disruption of phase between two polymorphic restriction sites. Phase is disrupted in approximately 1% of the PCR amplified material, possibly due to incomplete chain elongations and subsequent priming on the **heterologous** allele. Phase is disrupted in approximately 1/4 of cloned PCR fragments, possibly due to excision repair of heteroduplexes during cloning. The implications of these disruptions for the use of PCR in identifying **mutations** are discussed.

Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: Gene Amplification; *Heterozygote; *Isomerases--genetics--GE ; *Linkage (Genetics); *Methylmalonyl-CoA Mutase--genetics--GE; *Polymerase Chain Reaction; *Polymorphism, Restriction Fragment Length; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Base Sequence; Cell Line; Cloning, Molecular; Methyltransferases--metabolism--ME; Molecular Sequence Data; Restriction Mapping

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 5. (Isomerases); EC 5.4.99.2 (Methylmalonyl-CoA Mutase)

Record Date Created: 19901024

Record Date Completed: 19901024

11/9/4
DIALOG(R)File 155:MEDLINE(R)

06213951 PMID: 6309166
Effects of 5'deoxy-5'-methylthioadenosine on the metabolism of S-adenosyl methionine.
Dante R; Arnaud M; Niveleau A
Biochemical and biophysical research communications (UNITED STATES) Jul 18 1983, 114 (1) p214-21, ISSN 0006-291X Journal Code: 0372516
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The treatment of **transformed** rat cells with micromolar amounts of 5'deoxy 5'methyl thioadenosine induces rapid effects on the rate of methylation of DNA concomitantly with **alterations** of intracellular pools of S-adenosyl methionine and S-adenosyl homocysteine. Pulse chase labelling experiments indicate that 5'deoxy 5'methylthioadenosine does not inhibit the degradation of S-adenosyl homocysteine but inhibits the consumption of S-adenosyl methionine. In vitro transmethylation assays performed with **heterologous** DNA show that low doses of the thioethernucleoside do not significantly affect the DNA methyltransferase activity of cellular extracts. The biological role of 5'deoxy 5'methylthioadenosine, a natural molecule formed during the synthesis of polyamines is discussed.
Tags: Support, Non-U.S. Gov't
Descriptors: *Adenosine--analogs and derivatives--AA; *Deoxyadenosines; *S-Adenosylmethionine--metabolism--ME; *Thionucleosides--pharmacology--PD; Adenosine--pharmacology--PD; Animals; Cell Line; Cell **Transformation**, Neoplastic; DNA (Cytosine-5-)-Methyltransferase--metabolism--ME; Kidney --metabolism--ME; Kinetics; Rats; Sarcoma Viruses, Avian--genetics--GE; Sulfur Radioisotopes--diagnostic use--DU
CAS Registry No.: 0 (Deoxyadenosines); 0 (Sulfur Radioisotopes); 0 (Thionucleosides); 2457-80-9 (5'-methylthioadenosine); 29908-03-0 (S-Adenosylmethionine); 58-61-7 (Adenosine)
Enzyme No.: EC 2.1.1.37 (DNA (Cytosine-5-)-Methyltransferase)
Record Date Created: 19830909
Record Date Completed: 19830909
?logoff hold
01nov04 18:50:30 User228206 Session D2280.4
$5.71 1.784 DialUnits File155
$21.42 102 Type(s) in Format 9
$21.42 102 Types
$27.13 Estimated cost File155
$0.24 TELNET
$27.37 Estimated cost this search
$27.37 Estimated total session cost 1.784 DialUnits

Status: Signed Off. (1 minutes)

--enzymology--EN; Escherichia coli--genetics--GE; Genes, Structural, Bacterial; Molecular Sequence Data; **Mutation** ; Oligodeoxyribonucleotides; **Plasmids** ; Restriction Mapping; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Streptococcus pneumoniae--enzymology--EN ; Streptococcus pneumoniae--genetics--GE; Substrate Specificity
CAS Registry No.: 0 (DNA, Single-Stranded); 0 (Oligodeoxyribonucleotides); 0 (Plasmids)
Enzyme No.: EC 2.1.1.- (DNA modification methylase DpnA); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (endodeoxyribonuclease MboI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)
Record Date Created: 19900119
Record Date Completed: 19900119

8/9/68
DIALOG(R)File 155:MEDLINE(R)

08340869 PMID: 2684765
Nucleotide sequence of the FokI restriction-modification system: separate strand-specificity domains in the methyltransferase.
Looney M C; Moran L S; Jack W E; Feehery G R; Benner J S; Slatko B E; Wilson G G
New England Biolabs, Inc., Beverly, MA 01915.
Gene (NETHERLANDS) Aug 15 1989, 80 (2) p193-208, ISSN 0378-1119 Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The genes for FokI, a type-IIS restriction-modification system from Flavobacterium okeanokoites (asymmetric recognition sequence: 5'-GGATG/3'-CCTAC), were cloned into Escherichia coli. Recombinants **carrying** the fokIR and fokIM genes were found to modify their DNA completely, and to restrict lambdoid phages weakly. The nt sequences of the genes were determined, and the probable start codons were confirmed by aa sequencing. The FokI endonuclease (R.FokI) and methyltransferase (M.FokI) are encoded by single, adjacent genes, aligned in the same orientation, in the order M then R. The genes are large by the standards of type-II systems, 1.9 kb for the M gene, and 1.7 kb for the R gene. Preceding each gene is a pair of FokI recognition sites; it is conceivable that interactions between the sites and the FokI proteins could regulate expression of the genes. The aa sequences of the N- and C-terminal halves of M.FokI are similar to one another, and to certain other DNA- **adenine methyltransferases** , suggesting that the enzyme has a 'tandem' structure, such as could have arisen by the fusion of a pair of adjacent, ancestral M genes. Truncated derivatives of M. FokI were constructed by deleting the 5'- or 3'-ends of the fokIM gene. Deleting most of the C-terminus of M.FokI produced derivatives that methylated only the top (GGATG) strand of the recognition sequence. Conversely, deleting most of the N-terminus produced derivatives that methylated only the bottom (CATCC) strand of the recognition sequence. These results indicate that the domains in M.FokI for methylating the two strands of the recognition sequence are largely separate.
Descriptors: *Deoxyribonucleases, Type II Site-Specific--genetics--GE; *Flavobacterium--enzymology--EN; *Methyltransferases--genetics--GE; Amino Acid Sequence; Base Sequence; Binding Sites; Cloning, Molecular; DNA **Mutational** Analysis; Escherichia coli--genetics--GE; Flavobacterium --genetics--GE; Molecular Sequence Data; Substrate Specificity
Molecular Sequence Databank No.: GENBANK/M28828
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 3.1.21.- (endodeoxyribonuclease FokI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)
Record Date Created: 19891228
Record Date Completed: 19891228

different polypeptides MtaA, MtaB and MtaC of which MtaC harbors a corrinoid prosthetic group. The **heterologous** expression of mtaA and mtaB in Escherichia coli has been described previously. We report here on the overproduction of the apoprotein of MtaC in E. coli, on its reconstitution to the active holoprotein with either cob(II)alamin or methyl-cob(III)alamin, and on the properties of the reconstituted corrinoid protein. Reconstituted MtaC was found to contain 1 mol bound cobamide/mol. EPR spectroscopic evidence is presented for a His residue as an axial ligand to Co2+ of the bound corrinoid. This active-site His was identified by site-directed **mutagenesis** as His136 in the MtaC sequence that contains four His residues. The reconstituted MtaC, in the cob(I)amide oxidation state, was methylated with methanol in the presence of MtaB and demethylated with coenzyme M in the presence of MtaA. In the presence of both MtaB and MtaA, methyl-coenzyme M was formed from methanol and coenzyme M at specific rates comparable to those determined for the enzyme system purified from M. barkeri. M. barkeri contains an isoenzyme of MtaA designated MtbA. The isoenzyme reacted with MtaC with only 2.5% of the activity of MtaA.

Tags: Support, Non-U.S. Gov't

Descriptors: Adenosinetriphosphatase--chemistry--CH; *Adenosinetriphosphatase--metabolism--ME; * **Carrier** Proteins--chemistry--CH; * **Carrier** Proteins--metabolism--ME; *Histidine; *Methanosarcina barkeri--enzymology--EN; *Methyltransferases--chemistry--CH; *Methyltransferases--metabolism--ME; Adenosinetriphosphatase--isolation and purification--IP; Apoproteins--chemistry--CH; Apoproteins--metabolism--ME; Base Sequence; Binding Sites; **Carrier** Proteins--isolation and purification--IP; Cloning, Molecular; DNA Primers; Electron Spin Resonance Spectroscopy; Escherichia coli; Isoenzymes--chemistry--CH; Isoenzymes--isolation and purification--IP; Isoenzymes--metabolism--ME; Kinetics; Macromolecular Systems; Methyltransferases--isolation and purification--IP; **Mutagenesis**, Site-Directed; Protein Conformation; Recombinant Proteins--chemistry--CH; Recombinant Proteins--isolation and purification--IP; Recombinant Proteins--metabolism--ME

CAS Registry No.: 0 (Apoproteins); 0 (Carrier Proteins); 0 (DNA Primers); 0 (Isoenzymes); 0 (Macromolecular Systems); 0 (Mta72 protein); 0 (Recombinant Proteins); 71-00-1 (Histidine)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (methanol-2-mercaptoethanesulfonic acid methyltransferase); EC 3.6.1.3 (Adenosinetriphosphatase)

Record Date Created: 19980729

Record Date Completed: 19980729

11/9/2

DIALOG(R)File 155:MEDLINE(R)

12684915 PMID: 7607497

Expression of the SalI restriction-modification system of Streptomyces albus G in Escherichia coli.

Alvarez M A; Gomez A; Gomez P; Rodicio M R

Departamento de Biologia Funcional, Universidad de Oviedo, Spain.

Gene (NETHERLANDS) May 19 1995, 157 (1-2) p231-2, ISSN 0378-1119 Journal Code: 7706761

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The salIR and salIM genes of Streptomyces albus G encode the restriction endonuclease (ENase) and DNA methyltransferase (MTase) of the SalI restriction-modification (R-M) system. In S. albus G, the genes constitute an operon that is mainly transcribed from a promoter located upstream from salIR, the first gene of the operon. In addition, a second promoter, at the 3' end of salIR, allows independent transcription of the MTase gene. Expression of salIR and salIM in Escherichia coli was investigated. The ENase gene was not expressed in the **heterologous** host, probably due to

Record Date Completed: 19881206

8/9/77
DIALOG(R)File 155:MEDLINE(R)
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07909033 PMID: 3050986
The B- to Z-DNA equilibrium in vivo is perturbed by biological processes.
Zacharias W; Jaworski A; Larson J E; Wells R D
University of Alabama School of Medicine, Department of Biochemistry, Birmingham 35294.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Oct 1988, 85 (19) p7069-73, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: 2 P60 20614-10; PHS; GM-30822; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Right-handed B and left-handed Z conformations coexist in equilibrium in portions of **plasmids** in Escherichia coli. The equilibria are influenced by the length of the sequences that undergo the structural transitions and are perturbed by biological processes. The composite results of three types of determinations indicate a supercoil density of -0.025 in vivo. The coexistence of **alternative** DNA conformations in living cells implies the potential of these structures or their transitions for important functions in genetic regulatory processes.
Tags: Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.
Descriptors: *DNA--metabolism--ME; DNA, Bacterial--metabolism--ME; Escherichia coli--genetics--GE; Gene Expression Regulation; Nucleic Acid Conformation; **Plasmids** ; Protein Synthesis Inhibitors--pharmacology--PD; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME
CAS Registry No.: 0 (DNA, Bacterial); 0 (Plasmids); 0 (Protein Synthesis Inhibitors); 9007-49-2 (DNA)
Enzyme No.: EC 2.1.1.- (DNA modification methylase EcoRI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19881121
Record Date Completed: 19881121

8/9/78
DIALOG(R)File 155:MEDLINE(R)
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07870810 PMID: 2842672
A mutation **in the DNA adenine methylase gene (dam) of Salmonella typhimurium decreases susceptibility to 9-aminoacridine-induced frameshift** mutagenesis .
Ritchie L; Podger D M; Hall R M
CSIRO Division of Molecular Biology, North Ryde, NSW, Australia.
Mutation research (NETHERLANDS) Sep 1988, 194 (2) p131-41, ISSN 0027-5107 Journal Code: 0400763
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
A **mutant** of Salmonella typhimurium with a **reduced** response to **mutation** induction by 9-aminoacridine (9AA) has been isolated. The **mutation** (dam-2) is located in the DNA adenine methylase gene. The dam-2 **mutant** strain exhibits a level of sensitivity to 2-aminopurine (2AP) intermediate between that of the dam+ and the DNA adenine methylation-deficit dam-1 strain, and 2AP sensitivity was reversed by introduction of a mutH **mutation** or of the **plasmid** pMQ148 (which **carries** a functional Escherichia coli dam+ gene). However, the dam-2

strain is not grossly defective in DNA adenine methylase activity. Whole cell DNA appears full methylated at -GATC- sites. The levels of 9AA required to induce equivalent levels of frameshift **mutagenesis** in the dam-2 strain were approximately 2-fold higher than for the dam+ strain. Introduction of pMQ148 dam+ **reduced** the level of 9AA required for induction of frameshift **mutations** 4-fold in the dam-2 strain and 2-fold in the dam+ strain. The dam-2 **mutation** had no effect on the levels of ICR191 required for induction of frameshift **mutations** , but introduction of pMQ148 **reduced** the ICR191-induced **mutagenesis** 2-fold. The dam+/pMQ148, dam-2/pMQ148 and dam-1/pMQ148 strains showed identical dose-response curves for both 9AA and ICR191. These results are consistent with a slightly **reduced** (dam-2) or increased (pMQ148) rate of methylation at the replication fork. The 2AP sensitivity of the dam-2 strain cannot be simply explained. Furthermore, addition of methionine to the assay medium reverses the 2AP sensitivity of the dam-2 strain, but has no effect on 9AA **mutagenesis** .

Descriptors: Aminacrine--pharmacology--PD; *Aminoacridines--pharmacology --PD; *Genes, Bacterial; *Genes, Structural; *Methyltransferases--genetics --GE; * **Mutation** ; *Salmonella typhimurium--genetics--GE; DNA Transposable Elements; Genotype; Microbial Sensitivity Tests; Salmonella typhimurium --drug effects--DE; Salmonella typhimurium--enzymology--EN; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Species Specificity

CAS Registry No.: 0 (Aminoacridines); 0 (DNA Transposable Elements); 90-45-9 (Aminacrine)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19881004

Record Date Completed: 19881004

8/9/79

DIALOG(R)File 155:MEDLINE(R)

07730720 PMID: 2832849

Mutational **analysis of** insertion **sequence 50 (IS50) and transposon 5 (Tn5) ends.**

Makris J C; Nordmann P L; Reznikoff W S

Department of Biochemistry, University of Wisconsin-Madison 53706-1569.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Apr 1988, 85 (7) p2224-8, ISSN 0027-8424

Journal Code: 7505876

Contract/Grant No.: GM-19670; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Insertion sequence 50 (IS50) transposition utilizes a 19-base-pair "outside" end and a 19-base-pair "inside" end in inverted orientation relative to each other, whereas transposon 5 (Tn5) transposition utilizes two inverted outside ends. The frequency of transposition events that involve an inside end is regulated 1000-fold by the host dam methylase system. The end sequence requirements for transposition and its regulation by dam methylase were analyzed in Escherichia coli by generating random single base pair **mutations** in either an IS50 inside end or outside end placed in inverted orientation with respect to an unmutagenized outside end. The **mutations** were then isolated, assayed for transposition phenotype, and sequenced. **Mutations** were isolated at 15 of the 19 sites in the outside end. All of these **mutations** except those at position 4 decreased transposition. **Mutations** at position 4 (which is the only nonidentical base pair in a region of homology between the outside and inside ends) had no effect on transposition. **Mutations** were isolated at 11 of the 19 sites in the inside end. All of these **mutations** , including one at position 4, decreased transposition in dam- cells. **Mutations** at position 10 (within a dam recognition sequence) and 2 (not within a dam recognition sequence) **reduced** the magnitude of dam regulation. A

8/9/65

DIALOG(R)File 155:MEDLINE(R)

08427699 PMID: 2137369

Coregulation of the human O6-methylguanine-DNA methyltransferase with two unrelated genes that are closely linked.

Karran P; Stephenson C; Macpherson P; Cairns-Smith S; Priestley A

Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts., United Kingdom.

Cancer research (UNITED STATES) Mar 1 1990, 50 (5) p1532-7, ISSN 0008-5472 Journal Code: 2984705R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The **loss** of expression of the enzyme O6-methylguanine-DNA methyltransferase (the Mex- phenotype), which often results from cellular **transformation** , confers hypersensitivity to alkylating agents. We have observed two unrelated examples in which human cell lines have undergone a spontaneous **alteration** in their Mex phenotype during propagation in vitro. The change was reversible and was not the result of **mutation** . In both cases a **loss** of methyltransferase expression was accompanied by a simultaneous **loss** of expression of two metabolically unrelated enzymes: thymidine kinase and galactokinase. "Reversion" to methyltransferase expression was accompanied by simultaneous reexpression of both kinase activities. A third example of this coordinate gene regulation was seen with the Burkitt's lymphoma cell line Raji which expresses methyltransferase, thymidine kinase, and galactokinase at high levels. A thymidine kinase- Raji cell line derived by bromodeoxyuridine **mutagenesis** that is also Mex- was found to be galactokinase-. It appears that methyltransferase expression may in some instances be coordinately regulated with the tk and glk loci which are closely linked on human chromosome 17.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: *Galactokinase--biosynthesis--BI; *Gene Expression Regulation, Enzymologic; *Linkage (Genetics); *Methyltransferases--genetics --GE; *Thymidine Kinase--biosynthesis--BI; Adenosinetriphosphatase --analysis--AN; Adenosinetriphosphatase--biosynthesis--BI; Cell Line; Galactokinase--analysis--AN; Methyltransferases--biosynthesis--BI; O(6)-Met hylguanine-DNA Methyltransferase; Phenotype; Thymidine Kinase--analysis--AN

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.63 (O(6)-Methylguanine-DNA Methyltransferase); EC 2.7.1.21 (Thymidine Kinase); EC 2.7.1.6 (Galactokinase); EC 3.6.1.3 (Adenosinetriphosphatase)

Record Date Created: 19900321

Record Date Completed: 19900321

8/9/66

DIALOG(R)File 155:MEDLINE(R)

08367707 PMID: 2688642

A mutation in the dam gene of Vibrio cholerae: 2-aminopurine sensitivity with intact GATC methylase activity.

Bandyopadhyay R; Sengupta A; Das J

Biophysics Division, Indian Institute of Chemical Biology, Calcutta.

Biochemical and biophysical research communications (UNITED STATES) Dec 15 1989, 165 (2) p561-7, ISSN 0006-291X Journal Code: 0372516

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Vibrio cholerae **mutants** sensitive to 2-aminopurine (2AP) but with DNA adenine methylase activity similar to parental cells have been isolated. The **mutant** strains were sensitive to ultraviolet light (UV), methyl methane sulphonate (MMS) and 9-aminoacridine. The spontaneous **mutation** frequency of the **mutants** were not significantly affected. Attempts to isolate dam V. cholerae cells by screening 2AP sensitive cells have not been successful. All the **mutant** phenotypes could be suppressed by introducing the **plasmid** pRB103 **carrying** the dam gene of Escherichia coli into the **mutant** cells.

Tags: Support, Non-U.S. Gov't

Descriptors: 2-Aminopurine--pharmacology--PD; *Adenine --analogs and derivatives--AA; *Genes, Structural, Bacterial; * **Mutation** ; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; *Vibrio cholerae--genetics--GE; Aminacrine--pharmacology--PD; Bacteriophages --genetics--GE; Base Sequence; Conjugation, Genetic; Escherichia coli --genetics--GE; Kinetics; Methyl Methanesulfonate--pharmacology--PD; **Plasmids** ; Substrate Specificity; Ultraviolet Rays; Vibrio cholerae--drug effects--DE; Vibrio cholerae--radiation effects--RE

CAS Registry No.: 0 (Plasmids); 452-06-2 (2-Aminopurine); 66-27-3 (Methyl Methanesulfonate); 73-24-5 (Adenine); 90-45-9 (Aminacrine)

Enzyme No.: EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19900124

Record Date Completed: 19900124

8/9/67

08362911 PMID: 2687877

DpnA, a methylase for single-strand DNA in the Dpn II restriction system, and its biological function.

Cerritelli S; Springhorn S S; Lacks S A

Biology Department, Brookhaven National Laboratory, Upton, NY 11973.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 1989, 86 (23) p9223-7, ISSN 0027-8424 Journal Code: 7505876

Contract/Grant No.: GM29721; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The two DNA-adenine methylases encoded by the Dpn II restriction gene cassette were purified, and their activities were compared on various DNA substrates. DpnA was able to methylate single-strand DNA and double-strand DNA, whereas DpnM methylated only double-strand DNA. Although both enzymes act at 5'-GATC-3' in DNA, DpnA can also methylate sequences **altered** in the guanine position, but at a lower rate. A deletion **mutation** in the dpnA gene was constructed and transferred to the chromosome. Transmission by way of the **transformation** pathway of methylated and unmethylated **plasmids** to dpnA **mutant** and wild-type recipients was examined. The **mutant** cells restricted unmethylated donor **plasmid** establishment much more strongly than did wild-type cells. In the wild type, the single strands of donor **plasmid** DNA that enter by the **transformation** pathway are apparently methylated by DpnA prior to conversion of the **plasmid** to a double-strand form, in which the **plasmid** would be susceptible to the Dpn II endonuclease. The biological function of DpnA may, therefore, be the enhancement of **plasmid** transfer to Dpn II-containing strains of Streptococcus pneumoniae.

Tags: Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.

Descriptors: DNA, Single-Stranded--metabolism--ME; *Deoxyribonucleases, Type II Site-Specific--metabolism--ME; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME; Base Sequence; Deoxyribonucleases, Type II Site-Specific--genetics--GE; Escherichia coli

Thin Layer; DNA Damage--genetics--GE; Escherichia coli--genetics--GE; Gene Expression--genetics--GE; Methylation; Methylnitronitrosoguanidine --pharmacology--PD; Methyltransferases--metabolism--ME; **Plasmids** --genetics--GE; Restriction Mapping

CAS Registry No.: 0 (Plasmids); 443-72-1 (6-methyladenine); 70-25-7 (Methylnitronitrosoguanidine); 73-24-5 (Adenine)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Gene Symbol: dam

Record Date Created: 19920902

Record Date Completed: 19920902

8/9/53

DIALOG(R)File 155:MEDLINE(R)

09385101 PMID: 1639813

In vitro specificity of EcoRI DNA methyltransferase.

Reich N O; Olsen C; Osti F; Murphy J

Department of Chemistry, University of California, Santa Barbara 93106.

Journal of biological chemistry (UNITED STATES) Aug 5 1992, 267 (22) p15802-7, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The sequence selectivity of enzyme-DNA interactions was analyzed by comparing discrimination between synthetic oligonucleotides containing the canonical site GAATTC and **altered** DNA sequences with the EcoRI DNA methyltransferase. The specificities (kcat/KmDNA) are decreased from 5- to 23,000-fold relative to the unmodified site. For several substrates the decrease in kcat makes a disproportionate contribution to the specificity difference, suggesting that discrimination is mediated by the placement of critical catalytic residues rather than binding interactions. This is supported by our observation that specificity changes are generally not followed by changes in the stability of the methyltransferase-DNA complexes. Also, base pair substitutions near the site of methylation result in greater decreases in complex stability, suggesting that recognition and catalytic mechanisms overlap.

Tags: Comparative Study; Support, U.S. Gov't, Non-P.H.S.

Descriptors: Escherichia coli--enzymology--EN; *Oligodeoxyribonucleotides --metabolism--ME; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME; Base Sequence; Escherichia coli--genetics--GE; Kinetics; Mathematics; Models, Theoretical; Molecular Sequence Data; Oligodeoxyribonucleotides--chemical synthesis--CS; Oligodeoxyribonucleotides--isolation and purification--IP; **Plasmids** ; Restriction Mapping; Substrate Specificity

CAS Registry No.: 0 (Oligodeoxyribonucleotides); 0 (Plasmids)

Enzyme No.: EC 2.1.1.- (DNA modification methylase EcoRI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19920828

Record Date Completed: 19920828

8/9/54

DIALOG(R)File 155:MEDLINE(R)

09290515 PMID: 1579439

Artificial steroid hormone response element generated by dam-methylation.

Truss M; Bartsch J; Chalepakis G; Beato M

Institut fur Molekularbiologie und Tumorforschung, Marburg, Germany.

Nucleic acids research (ENGLAND) Apr 11 1992, 20 (7) p1483-6, ISSN 0305-1048 Journal Code: 0411011

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Using the interaction of steroid hormone receptors with their palindromic response elements as an example, we show here that cloning in dam+ bacterial strains can lead to artifactual results due to methylation of adenine residues at the N-6 position. Substitution of the T by an A in the third position of the half palindromes of the hormone responsive element TGTTCT(1) yields a functional element only when amplification is made in dam+ bacteria. **Mutant** palindromes methylated at the N-6 position of this adenine exhibit the same affinity for progesterone and glucocorticoid receptors as the consensus response element, whereas their unmethylated counterpart binds with negligible affinity. These observations underline the significance of hydrophobic interactions between receptors and the major groove of the DNA for discrimination among various responsive elements, and point to the importance of using dam- bacterial strains for the correct identification of the nucleotide sequence of cis-acting elements.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: DNA--metabolism--ME; *Methyltransferases--metabolism--ME; *Receptors, Steroid--metabolism--ME; *Regulatory Sequences, Nucleic Acid --genetics--GE; *Repetitive Sequences, Nucleic Acid--genetics--GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Base Sequence; Cloning, Molecular; Gene Expression Regulation--drug effects--DE; Methylation; Molecular Sequence Data; **Plasmids** --genetics--GE; Progesterone Congeners--pharmacology--PD; Receptors, Glucocorticoid --metabolism--ME; Receptors, Progesterone--metabolism--ME; Tumor Cells, Cultured

CAS Registry No.: 0 (Plasmids); 0 (Progesterone Congeners); 0 (Receptors, Glucocorticoid); 0 (Receptors, Progesterone); 0 (Receptors, Steroid); 9007-49-2 (DNA)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19920605
Record Date Completed: 19920605

8/9/55

DIALOG(R)File 155:MEDLINE(R)

09262616 PMID: 1563635

Overproduction, purification and characterization of M.HinfI methyltransferase and its deletion mutant .

Bassing C H; Kim Y G; Li L; Chandrasegaran S

Department of Environmental Health Sciences, Johns Hopkins University, School of Hygiene and Public Health, Baltimore, MD 21205.

Gene (NETHERLANDS) Apr 1 1992, 113 (1) p83-8, ISSN 0378-1119 Journal Code: 7706761

Contract/Grant No.: GM42140; GM; NIGMS

Erratum in Gene 1992 Nov 2;121(1) 183

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

We have used the polymerase chain reaction to **alter** transcriptional and translational signals surrounding the hinfIM gene [encoding M.HinfI methyltransferase (MTase)] so as to achieve overexpression in Escherichia coli. The PCR-generated hinfIM gene was subcloned in a high-expression **vector** under control of the hybrid trp-lac promoter. In addition, the positive retroregulator stem-loop sequence derived from the crystal protein-encoding gene of Bacillus thuringiensis was **inserted** downstream from hinfIM. Using a similar approach, we have also constructed

BsuBI--an isospecific restriction and modification system of PstI: characterization of the BsuBI genes and enzymes.

Xu G L; Kapfer W; Walter J; Trautner T A
Max-Planck-Institut fur Molekulare Genetik, Berlin, Germany.

Nucleic acids research (ENGLAND) Dec 25 1992, 20 (24) p6517-23, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The enzymes of the Bacillus subtilis BsuBI restriction/modification (R/M) system recognize the target sequence 5'CTGCAG. The genes of the BsuBI R/M system have been cloned and sequenced and their products have been characterized following overexpression and purification. The gene of the BsuBI DNA methyltransferase (M.BsuBI) consists of 1503 bp, encoding a protein of 501 amino acids with a calculated M(r) of 57.2 kD. The gene of the restriction endonuclease (R.BsuBI), comprising 948 bp, codes for a protein of 316 amino acids with a predicted M(r) of 36.2 kD. M.BsuBI modifies the adenine (A) residue of the BsuBI target site, thus representing the first A-N6-DNA methyltransferase identified in B. subtilis. Like R.PstI, R.BsuBI cleaves between the A residue and the 3' terminal G of the target site. Both enzymes of the BsuBI R/M system are, therefore, functionally identical with those of the PstI R/M system, encoded by the Gram **negative** species Providencia stuartii. This functional equivalence coincides with a pronounced similarity of the BsuBI/PstI DNA methyltransferases (41% amino acid identity) and restriction endonucleases (46% amino acid identity). Since the genes are also very similar (58% nucleotide identity), the BsuBI and PstI R/M systems apparently have a common evolutionary origin. In spite of the sequence conservation the gene organization is strikingly different in the two R/M systems. While the genes of the PstI R/M system are separated and transcribed divergently, the genes of the BsuBI R/M system are transcribed in the same direction, with the 3' end of the M gene overlapping the 5' end of the R gene by 17 bp.

Tags: Support, Non-U.S. Gov't
Descriptors: Bacillus subtilis--enzymology--EN; *Bacillus subtilis --genetics--GE; *Deoxyribonucleases, Type II Site-Specific--metabolism--ME; *Genes, Bacterial; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME; Amino Acid Sequence; Base Sequence; Chromosomes, Bacterial; Cloning, Molecular; DNA, Bacterial--genetics--GE; DNA, Bacterial--isolation and purification--IP; Deoxyribonucleases, Type II Site-Specific--genetics--GE; Escherichia coli--genetics--GE; Genomic Library; Methylation; Molecular Sequence Data; Oligodeoxyribonucleotides; **Plasmids** ; Restriction Mapping; Transcription, Genetic
Molecular Sequence Databank No.: GENBANK/L01541
CAS Registry No.: 0 (DNA, Bacterial); 0 (Oligodeoxyribonucleotides); 0 (Plasmids)
Enzyme No.: EC 2.1.1.- (DNA modification methylase PstI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.21.- (endodeoxyribonuclease PstI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)
Gene Symbol: BsuBI
Record Date Created: 19930210
Record Date Completed: 19930210

8/9/46

09496415 PMID: 1427082

Cloning, sequencing, overproduction, and purification of M. CviBI (GANTC) methyltransferase from Chlorella virus NC-1A [corrected]

Kan T N; Li L; Chandrasegaran S
Department of Environmental Health Sciences, Johns Hopkins University,

School of Hygiene and Public Health, Baltimore, MD 21205.
Gene (NETHERLANDS) Nov 2 1992, 121 (1) p1-7, ISSN 0378-1119 Journal Code: 7706761
Contract/Grant No.: GM 42140; GM; NIGMS
Erratum in Gene 1993 Apr 15;126(1) 159
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

We have cloned and sequenced the cvibIM gene from Chlorella virus NC-1A by selecting for the modification phenotype. The modification gene was cloned on a 7-kb BamHI fragment **inserted** into the BamHI site of the pUC13 **plasmid** . The cvibIM gene was localized at the 3' end of this fragment. Sequencing of this region revealed a large open reading frame that codes for methyltransferase (MTase; symbol M.) (predicting 260 amino acids). M.CviBI (GANTC) aa sequence is homologous to M.Dam(GATC), M.DpnII(GATC), and M.T4 (GATC), and not so to M.HinfI(GANTC), M.HhaII (GANTC), and M.DpnA(GATC). We also describe the use of the polymerase chain reaction technique to **alter** transcriptional and translational signals surrounding this gene so as to achieve overexpression in Escherichia coli. This construct yields M.CviBI at 2-3% of the total cellular protein. The MTase was purified by phosphocellulose, DEAE, and gel filtration chromatography. Its size by SDS-PAGE is approx. 28 kDa, in good agreement with that predicted from the nucleotide sequence.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Descriptors: Chlorella; *DNA Viruses--enzymology--EN; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics--GE; Amino Acid Sequence ; Base Sequence; Chromatography, DEAE-Cellulose; Chromatography, Gel; Cloning, Molecular; DNA Viruses--genetics--GE; DNA, Viral; Electrophoresis, Polyacrylamide Gel; Escherichia coli; Molecular Sequence Data; Open Reading Frames; **Plasmids** ; Polymerase Chain Reaction; Sequence Homology, Amino Acid; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--isolation and purification--IP; Transcription, Genetic; Translation, Genetic
Molecular Sequence Databank No.: GENBANK/M96366
CAS Registry No.: 0 (DNA, Viral); 0 (Plasmids)
Enzyme No.: EC 2.1.1.- (DNA modification methylase CviBI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Gene Symbol: cvibIM
Record Date Created: 19921211
Record Date Completed: 19921211

8/9/47
DIALOG(R)File 155:MEDLINE(R)

09491262 PMID: 1423294

Expression of the endogenous O6-methylguanine-DNA-methyltransferase protects Chinese hamster ovary cells from spontaneous G:C to A:T transitions.

Aquilina G; Biondo R; Dogliotti E; Meuth M; Bignami M
Laboratory of Comparative Toxicology and Ecotoxicology, Istituto Superiore di Sanita, Rome, Italy.
Cancer research (UNITED STATES) Dec 1 1992, 52 (23) p6471-5, ISSN 0008-5472 Journal Code: 2984705R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

We have investigated whether the presence of a DNA repair enzyme, O6-methylguanine-DNA-methyltransferase (MGMT), affects the nature of spontaneous **mutations** in a mammalian cell line. We compared spontaneous **mutations** in the adenine phosphoribosyl transferase gene of a Chinese hamster ovary (CHO) cell line that expressed 14,000 MGMT molecules/cell with those in the parental CHO cells lacking this DNA repair activity. The

10028247 PMID: 8145241

The Escherichia coli prr region encodes a functional type IC DNA restriction system closely integrated with an anticodon nuclease gene.

Tyndall C; Meister J; Bickle T A
Department of Microbiology, Basel University, Switzerland.

Journal of molecular biology (ENGLAND) Apr 1 1994, 237 (3) p266-74, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The prr locus was originally described as coding a ribonuclease that is activated after phage T4 infection to cut within the anticodon of a specific tRNA, inactivating protein synthesis and thus blocking phage development. Wild-type T4 phage has two genes coding the enzymes polynucleotide kinase and RNA ligase, whose only function seems to be to repair the damage done by the anticodon nuclease. As the only apparent function of the prr ribonuclease is to combat phage infection, it can be considered as an RNA-based restriction enzyme. In non-infected cells, the prr enzyme is kept inactive in a complex with three other proteins which were predicted on the basis of DNA homologies to be the subunits of a type IC DNA restriction and modification system. Unlike other type IC systems so far characterized, prr is chromosomally rather than **plasmid** coded. However, sequences upstream from prr also have homology with sequences from the **plasmid** R124 and the prophage P1. We have now investigated the prr system and shown that it is indeed a bona fide type IC system which we call EcoprrI, and which is active both in vivo and in vitro. The system is fully functional even in the absence of the anticodon nuclease and seems to be a typical type I enzyme. EcoprrI recognizes the sequence CCA(N7)RTGC. One peculiarity is that, with low efficiency, EcoprrI will recognize and methylate variants of its recognition sequence such as CCT(N7)ATGC, which is methylated in one strand of the DNA only.

Tags: Support, Non-U.S. Gov't
Descriptors: DNA Modification Methylases--genetics--GE; *Escherichia coli --genetics--GE; *Genes, Bacterial--genetics--GE; *Ribonucleases--genetics --GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--genetics --GE; Amino Acid Sequence; Bacteriophage lambda--growth and development--GD ; Base Sequence; Cloning, Molecular; Cosmids; DNA Modification Methylases --chemistry--CH; DNA Modification Methylases--isolation and purification --IP; DNA Modification Methylases--metabolism--ME; DNA, Viral--metabolism --ME; Methylation; Molecular Sequence Data; **Mutagenesis , Insertional** ; Restriction Mapping; Sequence Analysis, DNA; Sequence Homology, Amino Acid; Sequence Homology, Nucleic Acid; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--chemistry--CH; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--isolation and purification--IP; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME
Molecular Sequence Databank No.: GENBANK/X75452
CAS Registry No.: 0 (Cosmids); 0 (DNA, Viral)
Enzyme No.: EC 2.1.1.- (DNA Modification Methylases); EC 2.1.1.- (DNA modification methylase EcoprrI); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)); EC 3.1.- (Ribonucleases); EC 3.1.- (anticodon nuclease)
Gene Symbol: hsdM; hsdR; hsdS; prr
Record Date Created: 19940429
Record Date Completed: 19940429

8/9/36

10004952 PMID: 8125341

The DNA adenine methyltransferase **-encoding gene (dam) of Vibrio cholerae.**

Bandyopadhyay R; Das J
Biophysics Division, Indian Institute of Chemical Biology, Calcutta.
Gene (NETHERLANDS) Mar 11 1994, 140 (1) p67-71, ISSN 0378-1119
Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Vibrio Cholera

The DNA **adenine methyltransferase** (MTase)-encoding gene (dam) of Vibrio cholerae, an organism belonging to the family Vibrionaceae, has been cloned and the complete nucleotide (nt) sequence determined. V. cholerae dam encodes a 21.5-kDa protein and is directly involved in methyl-directed DNA mismatch repair. It can substitute for the Escherichia coli enzyme and can suppress the phenotypic traits associated with E. coli dam **mutants** . Overproduction of V. cholerae Dam MTase does not result in hypermutability in either V. cholerae or E. coli cells. Overproduction of V. cholerae Dam in a pUC **plasmid** , however, fails to suppress the 2-aminopurine (2-AP)-sensitive phenotype of E. coli dam **mutants** . Homology between the nt and deduced amino acid (aa) sequences of the E. coli and V. cholerae dam genes is only 30-35%.

Tags: Support, Non-U.S. Gov't
Descriptors: Methyltransferases--genetics--GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); *Vibrio cholerae--genetics--GE; Amino Acid Sequence; Base Sequence; Cloning, Molecular; DNA, Bacterial; Escherichia coli; Genetic Complementation Test; Genetic **Vectors** ; Molecular Sequence Data; Recombinant Proteins--genetics--GE; Vibrio cholerae--enzymology--EN
Molecular Sequence Databank No.: GENBANK/X67820
CAS Registry No.: 0 (DNA, Bacterial); 0 (Genetic Vectors); 0 (Recombinant Proteins)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Gene Symbol: dam
Record Date Created: 19940414
Record Date Completed: 19940414

8/9/37
DIALOG(R)File 155:MEDLINE(R)

09957901 PMID: 8294016
Cloning of the YenI restriction endonuclease and methyltransferase from Yersinia enterocolitica serotype O8 and construction of a transformable **R-M+** mutant .
Kinder S A; Badger J L; Bryant G O; Pepe J C; Miller V L
Department of Microbiology and Molecular Genetics, University of California, Los Angeles 90024.
Gene (NETHERLANDS) Dec 22 1993, 136 (1-2) p271-5, ISSN 0378-1119
Journal Code: 7706761
Contract/Grant No.: GM-07104; GM; NIGMS; K11 DE-00212; DE; NIDCR; RO1 AI-27342; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Two different clonal groups of pathogenic Yersinia enterocolitica strains, American and non-American, have been recognized. These are distinguished by a number of criteria, including their virulence in a murine model of infection. However, genetic analysis of virulence in American strains has been hampered due to the severe restriction of **transformed** or electroporated DNA. Thus, we cloned the yenIMR locus from the American serotype strain 8081c, which encodes YenI, an isoschizomer of PstI. This clone encodes both the restriction endonuclease and

First Hit

L26: Entry 27 of 74 File: PGPB Jul 4, 2002

PGPUB-DOCUMENT-NUMBER: 20020086332
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020086332 A1

TITLE: Method of reducing bacterial proliferation

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Mahan, Michael J.	Santa Barbara	CA	US	
Heithoff, Douglas M.	Goleta	CA	US	
Low, David A.	Goleta	CA	US	
Sinsheimer, Robert L.	Santa Barbara	CA	US	

US-CL-CURRENT: 435/7.1

CLAIMS:

That which is claimed is:

1. A method of reducing bacterial virulence, comprising: contacting bacteria with an agent that alters the bacteria's native level of DNA methyltransferase (Dam) activity thereby altering the bacteria's native level of methylation of adenine in a GATC tetranucleotide of the bacteria, and thereby inhibiting virulence of the bacteria.

2. The method of claim 1, wherein the agent reduces the bacteria's native level of DNA methyltransferase activity.

3. The method of claim 1, wherein the agent reduces the Dam activity by reducing the bacteria's level of expression of Dam.

4. The method of claim 1, wherein the agent reduces the Dam activity by blocking a Dam interaction site.

5. The method of claim 1, wherein the agent increases the bacteria's native level of DNA methyltransferase activity.

6. The method of claim 1, wherein the agent reduces the bacteria's native level of methylated adenine in a GATC tetranucleotide by inhibiting DNA methyltransferase activity.

7. The method of claim 1, wherein the agent increases the bacteria's native level of methylated adenine in a GATC tetranucleotide by increasing DNA methyltransferase activity.

8. The method of claim 1, wherein the agent binds a Dam enzyme.

such as CDC in maize (Colasanti et al., 1991) the WT gene (Farmer et al., 1994) and P68 (Chen et al., 1991). In an illustrative embodiment, the DAM-methylase gene, the expression product of which catalyzes methylation of adenine residues in the DNA of the plant, is used. Methylated adenines will not affect cell viability and will be found only in the tissues in which the DAM-methylase gene is expressed, because such methylated residues are not found endogenously in plant DNA. A suitable system for DNA binding is the lexA-C1 system. Generally, the construct is exogenous and includes suitable promoters.

Detailed Description Text (152):
Brooks, J. E., Blumenthal, R. M., and Gingeras, T. R., (1993). The isolation and characterization of the Escherichia coli DNA adenine methylase (DAM) gene. Nucl Acids Res. 11:837-851.

Other Reference Publication (3):
Brooks et al. "The Isolation and Characterization of the Escherichia coli DNA Adenine Methylase (dam) Gene," Nucl. Acids Res. 11(3):837-51 (1983).

CLAIMS:

8. The recombinant DNA molecule of claim 1, wherein said gene product is a DAM methylase.

Previous Doc Next Doc Go to Doc#

The method of claim 3, wherein said methylase gene is a DAM methylase gene.

DOCUMENT-IDENTIFIER: US 5856090 A
TITLE: DNA-methylase linking reaction

Detailed Description Text (37):
The cytosine-specific Nla III methylase is described in D. Labbe et al., "Cloning and Characterization of Two Tandemly Arranged DNA Methyltransferase Genes of Neisseria lactamica: An Adenine-Specific M.NlaIII and a Cytosine-Type Methylase," Mol. Gen. Genet. 224: 101-110 (1990). The Nla III cytosine-specific methylase recognizes the sequence C-A-T-G, with residue 1, the only cytosine, being methylated.

L2: Entry 2 of 17 File: USPT Aug 28, 2001

DOCUMENT-IDENTIFIER: US 6281348 B1
TITLE: Reversible nuclear genetic system for male sterility in transgenic plants

Brief Summary Text (16):
Methylation patterns are established by methods such as the use of methyl-sensitive CpG-containing promoters (genes). In general, actively transcribed sequences are under methylated. In animals, sites of methylation are modified at CpG sites (residues). Genetic control of methylation of adenine (A) and cytosine (C) (nucleotides present in DNA) is affected by genes in bacterial and mammalian species. In plants, however, methyl moieties exist in the sequence CXG, where X can be A, C or T, where C is the methylated residue. Inactivation due to methylation of A is not known in plants, particularly within GATC sites known to be methylated in other systems.

Brief Summary Text (18):
Envisioning directed methylation as a means for control of plant development, for example, to effect male sterility, would be discouraged by difficulties anticipated in using expression of a gene that has a generalized inactivating effect in a ubiquitous target, e.g., a methylase gene such as the E. coli DNA adenine methylase (DAM) for which GATC is a target, as a means to control a specific developmental step without otherwise deleteriously affecting the plant. The DAM target exists in many promoters, therefore, a problem of maintaining plant viability would be expected from inactivating promoters and/or genes that are crucial for cell viability. Unless there was a way to "compartmentalize" methylation introduced into a host system by an exogenous vector, methylation as an approach to producing male sterility by genetic means would not be expected to succeed. The present invention provides methods and compositions to compartmentalize and to manipulate genes such as DAM to effect changes in plant development.

Brief Summary Text (61):
To produce a male-sterile plant, the promoter allows gene expression only in a specific tissue, preferably a tissue critical for pollen formation or function, such as in the tapetum, in the anther or in early microspores. The construct may also include a methylase gene as the DNA sequence encoding a gene product capable of inhibiting pollen formation or function. A suitable methylase gene is a bacterial DAM (DNA adenine methylating) gene. Bacterial sources include E. coli. The The DAM class of genes methylates a N6 position of adenine in the nucleotide sequence GATC. The construct includes a target DNA and is dominant negative because it represses the synthesis of MRNA by the target DNA.

Detailed Description Text (2):
The present invention relates the use of a genetic construct which includes a transcriptional activator and gene capable of acting on a DNA binding site to activate a dominant negative gene, a dominant negative gene, and suitable promoters, including a tissue-specific promoter controlling a gene acting on a DNA binding site, to affect plant development, for example, to cause male sterility. In transgenic plants, suitable dominant negative genes include cytotoxin genes, methylase genes, growth-inhibiting genes. Dominant negative genes include diphtheria toxin A-chain gene (Czako and An, 1991), cell cycle division mutants

First Hit

L26: Entry 1 of 74 File: PGPB Oct 21, 2004

DOCUMENT-IDENTIFIER: US 20040209257 A1
TITLE: Method for cloning and expression of AcuI restriction endonuclease and AcuI methylase in E. coli

Summary of Invention Paragraph:
[0011] There are three major groups of DNA methyltransferases based on the position and the base that is modified (C5-cytosine methylases, N4-cytosine methylases, and N6-adenine methylases). N4-cytosine and N6-adenine methylases are amino-methyltransferases (Malone et al. J. Mol. Biol. 253:618-632 (1995)). When a restriction site on DNA is modified (methylated) by the methylase, it is resistant to digestion by the cognate restriction endonuclease. Sometimes methylation by a non-cognate methylase can also confer DNA sites resistant to restriction digestion. For example, Dcm methylase modification of 5' CCWGG 3' (W=A or T) can also make the DNA resistant to PspGI restriction digestion. Another example is that CpG methylase can modify the CG dinucleotide of the NotI site (5' GCGGCCGC 3') and make it refractory to NotI digestion (New England Biolabs' (Beverly, Mass.) catalog, 2002-03, page 252). Therefore methylases can be used as a tool to modify certain DNA sequences and make them uncleavable by restriction enzymes.

First Hit

L26: Entry 6 of 74 File: PGPB Apr 1, 2004

DOCUMENT-IDENTIFIER: US 20040063126 A1
TITLE: Electrochemical sensor using intercalative, redox-active moieties

Detail Description Paragraph:
[0198] Garcia, R. A., Bustamante, C. J. & Reich, N. O. Sequence-specific recognition by cytosine C-5 and adenine N-6 DNA methyltransferases requires different deformations of DNA. P. Natl. Acad. Sci. USA 93, 7618-7622 (1996).

First Hit

Full - [FULL]
Title - [TI]
Citation - [CIT]
Front - [FRO]
Review - [REV]
Classification - [CLS]
Date - [DATE]
Reference - [REF]
Sequences - [SEQ]
Attachments - [ATT]
Claims - [CLM]
KWIC - [KWIC]
Drwg Desc - [DRAW]
Image - [IMG]

L26: Entry 23 of 74 File: PGPB Oct 10, 2002

DOCUMENT-IDENTIFIER: US 20020146716 A1
TITLE: Electrochemical sensor using intercalative, redox-active moieties

Detail Description Paragraph:

[0188] Garcia, R. A., Bustamante, C. J. & Reich, N. O. Sequence-specific recognition by cytosine C-5 and adenine N-6 DNA methyltransferases requires different deformations of DNA. P. Natl. Acad. Sci. USA 93, 7618-7622 (1996).

First Hit

L26: Entry 33 of 74 File: PGPB Feb 28, 2002

DOCUMENT-IDENTIFIER: US 20020025515 A1
TITLE: Inviable virus particles as scaffolds for constructing flexible detection systems

Detail Description Paragraph:
[0069] Type II DNA methyltransferases (Mtases) transfers a methyl group to a cytosine or adenine base residue in a double stranded DNA sequence. A DNA Mtase can either stably or covalently bind to a target DNA sequence under appropriate conditions. For example, if the C5 position of a cytosine base carries a fluorine instead of a hydrogen, certain DNA Mtases can remain permanently and covalently attached to the cytosine upon binding. For another example, the DNA Mtase may stably associate with a target DNA sequence if one uses an analogue of the normal cofactor S-adenosylmethionine (SAM), Sinefungin, or a DNA comprising 5-azacytosine (a cytosine analogue). A stable Mtase-DNA complex may also be formed if the target sequence comprises a mismatched cytosine or if one uses a suitable mutant Mtase.

DOCUMENT-IDENTIFIER: US 6764843 B2
TITLE: Method of cloning and expression of BsmBI restriction endonuclease and BsmBI methylase in E. coli and purification of BsmBI endonuclease

Brief Summary Text (13):
There are three major groups of DNA methyltransferases based on the position and the base that is modified (C5 cytosine methylases, N4 cytosine methylases, and N6 adenine methylases). N4 cytosine and N6 adenine methylases are amino-methyltransferases (Malone et al. J. Mol. Biol. 253:618-632, (1995)). When a restriction site on DNA is modified (methylated) by the methylase, it is resistant to digestion by the cognate restriction endonuclease. Sometimes methylation by a non-cognate methylase can also confer the DNA site resistant to restriction digestion. For example, Dcm methylase modification of 5'CCWGG3' (SEQ ID NO:8) (W=A or T) can also make the DNA resistant to PspGI restriction digestion. Another example is that CpM methylase can modify the CG dinucloetide and make the NotI site (5'GCGGCCGC3' (SEQ ID NO:9)) refractory to NotI digestion (New England Biolabs' Catalog, 2000-01, page 220). Therefore methylases can be used as a tool to modify certain DNA sequences and make them uncleavable by restriction enzymes.

07288433 PMID: 2430942

Methylation-dependent transcription controls plasmid **replication of the CloDF13 cop-1(Ts)** mutant .

van Putten A J; de Lang R; Veltkamp E; Nijkamp H J; Van Solingen P; van den Berg J A

Journal of bacteriology (UNITED STATES) Nov 1986, 168 (2) p728-33, ISSN 0021-9193 Journal Code: 2985120R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The CloDF13 cop-1(Ts) **mutant** expresses a temperature-dependent **plasmid** copy number. At 42 degrees C the **mutant** shows a "runaway" behavior, and cells harboring this **plasmid** are killed. The cop-1(Ts) **mutation** is a G-to-A transition that disturbs one of the two methylation sites which are located opposite in the stem-loop structure within a region involved in both the initiation of primer synthesis for DNA replication and the termination of the cloacin operon transcript. We demonstrate that the **mutation** results in an increased primer (RNA II) synthesis resulting from nonconditional enhanced RNA II promoter activity, which at 42 degrees C causes a decrease in the amount of active replication repressor molecules (RNA I) synthesized from the opposite strand. We found that the absence of Dam methylation abolishes the **mutant** phenotype and that under this condition the high **mutant** level of RNA II synthesis is **reduced** , which is accompanied by a restoration of the regulation by RNA I. The role of methylation in the regulation of **plasmid** replication is discussed.

Tags: Support, Non-U.S. Gov't

Descriptors: DNA Replication; *Methyltransferases--metabolism--ME; * **Plasmids** ; *Transcription, Genetic; Cloacin--genetics--GE; Escherichia coli--genetics--GE; Methylation; **Mutation** ; Promoter Regions (Genetics); RNA, Bacterial--genetics--GE; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Temperature; Terminator Regions (Genetics)

CAS Registry No.: 0 (Plasmids); 0 (RNA, Bacterial); 37370-19-7 (Cloacin)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19861224

Record Date Completed: 19861224

13953474 PMID: 9654068

Methanol:coenzyme M methyltransferase from Methanosarcina barkeri--identification of the active-site histidine in the corrinoid-harboring subunit MtaC by site-directed mutagenesis .

Sauer K; Thauer R K

Max-Planck-Institut fur terrestrische Mikrobiologie and Laboratorium fur Mikrobiologie des Fachbereichs Biologie der Philipps-Universitat, Marburg, Germany.

European journal of biochemistry / FEBS (GERMANY) May 1 1998, 253 (3) p698-705, ISSN 0014-2956 Journal Code: 0107600

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The enzyme system catalyzing the formation of methyl-coenzyme M from methanol and coenzyme M in Methanosarcina barkeri is composed of the three different polypeptides MtaA, MtaB and MtaC of which MtaC harbors a corrinoid prosthetic group. The **heterologous** expression of mtaA and mtaB in Escherichia coli has been described previously. We report here on the overproduction of the apoprotein of MtaC in E. coli, on its reconstitution to the active holoprotein with either cob(II)alamin or methyl-cob(III)alamin, and on the properties of the reconstituted corrinoid protein. Reconstituted MtaC was found to contain 1 mol bound cobamide/mol. EPR spectroscopic evidence is presented for a His residue as an axial ligand to Co2+ of the bound corrinoid. This active-site His was identified by site-directed **mutagenesis** as His136 in the MtaC sequence that contains four His residues. The reconstituted MtaC, in the cob(I)amide oxidation state, was methylated with methanol in the presence of MtaB and demethylated with coenzyme M in the presence of MtaA. In the presence of both MtaB and MtaA, methyl-coenzyme M was formed from methanol and coenzyme M at specific rates comparable to those determined for the enzyme system purified from M. barkeri. M. barkeri contains an isoenzyme of MtaA designated MtbA. The isoenzyme reacted with MtaC with only 2.5% of the activity of MtaA.

Tags: Support, Non-U.S. Gov't

Descriptors: Adenosinetriphosphatase--chemistry--CH; *Adenosinetriphosphatase--metabolism--ME; * **Carrier** Proteins--chemistry--CH; * **Carrier** Proteins--metabolism--ME; *Histidine; *Methanosarcina barkeri--enzymology--EN; *Methyltransferases--chemistry--CH; *Methyltransferases--metabolism--ME; Adenosinetriphosphatase--isolation and purification--IP; Apoproteins--chemistry--CH; Apoproteins--metabolism--ME; Base Sequence; Binding Sites ; **Carrier** Proteins--isolation and purification--IP; Cloning, Molecular; DNA Primers; Electron Spin Resonance Spectroscopy; Escherichia coli; Isoenzymes--chemistry--CH; Isoenzymes--isolation and purification--IP; Isoenzymes--metabolism--ME; Kinetics; Macromolecular Systems; Methyltransferases--isolation and purification--IP; **Mutagenesis** , Site-Directed; Protein Conformation; Recombinant Proteins--chemistry--CH; Recombinant Proteins--isolation and purification--IP; Recombinant Proteins--metabolism--ME

06015635 PMID: 6336742

Phenotypic reversal in dam mutants **of Escherichia coli K-12 by a recombinant** plasmid **containing the dam+ gene.**

Arraj J A; Marinus M G

Journal of bacteriology (UNITED STATES) Jan 1983, 153 (1) p562-5, ISSN 0021-9193 Journal Code: 2985120R

Contract/Grant No.: GM22055; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A recombinant **plasmid** , pMQ3, **carrying** the dam gene of Escherichia coli K-12, was constructed and **transformed** into dam+ and dam- strains. Both dam- and dam+ strains containing pMQ3 showed a wild phenotype for all traits, including **mutation** rate, except for a 10-fold increase in DNA adenine methylase activity.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: DNA (Cytosine-5-)-Methyltransferase--genetics--GE; *Escherichia coli--genetics--GE; *Genes, Bacterial; *Methyltransferases--genetics--GE; * **Transformation** , Bacterial; Cloning, Molecular; DNA, Recombinant; Escherichia coli--enzymology--EN; **Mutation** ; Phenotype; **Plasmids** ; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)

CAS Registry No.: 0 (DNA, Recombinant); 0 (Plasmids)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.37 (DNA (Cytosine-5-)-Methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19830225

Record Date Completed: 19830225

6306572 PMID: 6316110

Insertion mutations **in the dam gene of Escherichia coli K-12.**

Marinus M G; Carraway M; Frey A Z; Brown L; Arraj J A

Molecular & general genetics - MGG (GERMANY, WEST) 1983, 192 (1-2) p288-9, ISSN 0026-8925 Journal Code: 0125036

Contract/Grant No.: GM30330; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The dam gene of E. coli can be inactivated by **insertion** of Tn9 or Mud phage. Strains bearing these **mutations** are viable indicating that the dam gene product is dispensable.

Tags: Comparative Study; Support, U.S. Gov't, P.H.S.

Descriptors: *Escherichia coli--genetics--GE; *Genes, Bacterial; *Methyltransferases--genetics--GE; DNA Transposable Elements; **Mutation** ; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)

CAS Registry No.: 0 (DNA Transposable Elements)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19840127

Record Date Completed: 19840127

06546843 PMID: 6376282

Correlation of DNA adenine methylase activity with spontaneous mutability in Escherichia coli K-12.

Marinus M G; Poteete A; Arraj J A

Gene (NETHERLANDS) Apr 1984, 28 (1) p123-5, ISSN 0378-1119
Journal Code: 7706761
Contract/Grant No.: GM30330; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Using a multicopy **plasmid** in which the tac promoter has been placed in front of the dam gene of Escherichia coli K-12, we show that levels of DNA adenine methylase activity are correlated with the spontaneous **mutation** frequency.

Tags: Support, U.S. Gov't, P.H.S.
Descriptors: Bacterial Proteins--genetics--GE; *Escherichia coli --genetics--GE; *Methyltransferases--genetics--GE; * **Mutation** ; Bacterial Proteins--physiology--PH; DNA Repair; DNA, Bacterial--metabolism--ME; Methyltransferases--physiology--PH; **Plasmids** ; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)
CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA, Bacterial); 0 (Plasmids)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))
Record Date Created: 19840727
Record Date Completed: 19840727

06968320 PMID: 3905517

Nucleotide sequence of the ksgA gene of Escherichia coli: comparison of methyltransferases **effecting dimethylation of** adenosine **in ribosomal RNA.**

van Buul C P; van Knippenberg P H

Gene (NETHERLANDS) 1985, 38 (1-3) p65-72, ISSN 0378-1119
Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The ksgA gene of Escherichia coli encodes a methyltransferase (MeT) that specifically dimethylates two adjacent adenosines near the 3' end of 16S RNA in the 30S particle. Its inactivation leads to kasugamycin (Ksg) resistance. Several **plasmids** were constructed with **inserts** which complemented chromosomal ksgA **mutations** . One of these **inserts** was sequenced and found to contain an open reading frame (ORF) sufficient to code for the previously identified 30-kDal MeT. We have compared the amino acid (aa) sequence of the ksgA-encoded enzyme with three published sequences of MeT involved in dimethylation of an adenosine residue in 23S RNA and rendering the organisms resistant to the MLS antibiotics. The homologous patches in the sequences of all four enzymes suggest that those might correspond to contact points for the common substrates, e.g., for the adenosine residue(s) and S-adenosylmethionine (SAM).

Tags: Comparative Study
Descriptors: *Aminoglycosides; *Anti-Bacterial Agents; *Escherichia coli --genetics--GE; *Genes, Bacterial; *Methyltransferases--genetics--GE; *RNA, Ribosomal--metabolism--ME; Amino Acid Sequence; Base Sequence; Cloning, Molecular; Codon; Drug Resistance, Microbial; Gene Expression Regulation; Methylation; Methyltransferases--metabolism--ME
Molecular Sequence Databank No.: GENBANK/M11054
CAS Registry No.: 0 (Aminoglycosides); 0 (Anti-Bacterial Agents); 0 (Codon); 0 (RNA, Ribosomal); 6980-18-3 (kasugamycin)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.48 (rRNA(**adenine** -N6)- **methyltransferase**)
Record Date Created: 19860121
Record Date Completed: 19860121

07050281 PMID: 3512529

Direct role of the Escherichia coli Dam DNA methyltransferase in methylation-directed mismatch repair.

Schlagman S L; Hattman S; Marinus M G

Journal of bacteriology (UNITED STATES) Mar 1986, 165 (3) p896-900, ISSN 0021-9193 Journal Code: 2985120R

Contract/Grant No.: GM29227; GM; NIGMS; GM30330; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The T4 dam+ gene has been cloned (S. L. Schlagman and S. Hattman, Gene 22:139-156, 1983) and transferred into an Escherichia coli dam-host. In this host, the T4 Dam DNA methyltransferase methylates mainly, if not exclusively, the sequence 5'-GATC-3'; this sequence specificity is the same as that of the E. coli Dam enzyme. Expression of the cloned T4 dam+ gene suppresses almost all the phenotypic traits associated with E. coli dam **mutants** , with the exception of hypermutability. In wild-type hosts, 20- to 500-fold overproduction of the E. coli Dam methylase by **plasmids** containing the cloned E. coli dam+ gene results in a hypermutability phenotype (G.E. Herman and P. Modrich, J. Bacteriol. 145:644-646, 1981; M.G. Marinus, A. Poteete, and J.A. Arraj, Gene 28:123-125, 1984). In contrast, the same high level of T4 Dam methylase activity, produced by **plasmids** containing the cloned T4 dam+ gene, does not result in hypermutability. To account for these results we propose that the E. coli Dam methylase may be directly involved in the process of methylation-instructed mismatch repair and that the T4 Dam methylase is unable to substitute for the E. coli enzyme.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: *DNA Repair; *DNA, Bacterial--metabolism--ME; *Escherichia coli--enzymology--EN; *Methyltransferases--metabolism--ME; Cloning, Molecular; Escherichia coli--genetics--GE; Methylation; Methyltransferases --genetics--GE; **Mutation** ; Phenotype; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Substrate Specificity; T-Phages--enzymology--EN; T-Phages--genetics--GE

CAS Registry No.: 0 (DNA, Bacterial)

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L9: Entry 21 of 41 File: USPT Dec 15, 1998

DOCUMENT-IDENTIFIER: US 5849305 A
TITLE: Construction of Pasteurella haemolytica vaccines

Detailed Description Text (2):
It is a discovery of the present invention that P. haemolytica contains at least one restriction-modification system, called herein the PhaI system. Both the restriction endonuclease and the methyltransferase have been molecularly cloned. One such molecular clone (E. coli PhaIMtase) has been deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md., 20852, USA, on Dec. 2, 1993, under the terms of the Budapest Treaty as Accession No. ATCC 69500. A preliminary sequence of the methyltransferase gene has been determined. The predicted amino acid sequence of the methyltransferase contains sequence motifs which are consistent with an adenine-methylating specificity.

Detailed Description Text (5):
It has also been discovered by the present inventors, that a barrier to transformation of P. haemolytica can be overcome by treating DNA with a methylating enzyme, such as the PhaI methyltransferase. Such enzymes modify DNA substrates such that endonucleases which recognize 5'-GATGC-3' or 5'-GCATC-3' sequences are inhibited in their ability to digest such modified substrates. Examples of such endonucleases are PhaI endonuclease and SfaNI endonuclease. While applicants do not wish to be bound by any particular hypothesis on the mechanism of action of such methyltransferase enzymes, it appears that the PhaI methyltransferase methylates specific adenine residues in DNA.

Detailed Description Text (50):
The possibility that a system similar to E. coli mcr, mrr, is active in P. haemolytica was investigated by passage of pPh.DELTA.roACm.sup.R pD80 through E. coli strain GM2163 previously transformed with the recombinant cosmid containing PhaI methyltransferase (Raleigh et al., Proc. Natl. Acad. Sci. 83:9070-9074 (1986)). Since strain GM2163 is dam-, the resultant DNA would only be modified at PhaI sites (Marinus et al., Mol. Gen. Genet. 192:288-289 (1983)). Efficiency of transformation with this DNA, however, was not substantially different than that using DNA obtained from PhaI Mtase which is dam-methylated (Table 1). It is possible a second restriction system, not readily detectable in cell extracts, is active in P. haemoytica A1. Genes have been described in Neisseria gonnorhea MS11 which encode for restriction enzymes which are expressed at levels too low to detect biochemically (Stein et al., J. Bact. 74:4899-4906 (1992).

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1: Microbiologia. 1994 Dec;10(4):357-70. Related

Entrez PubMed

New methods in Salmonella genetics.

Casadesus J, Flores A, Beuzon CR, Torreblanca J, M Cano DA.

Departamento de Genetica, Universidad de Sevilla.

This review summarizes several recent developments in genetics; some of the procedures described can be easi Escherichia coli and have also potential applications in n bacteria. The novel methods outlined include genetic ma procedures, ancillary tools for cloning, a strategy for an DNA-protein interactions in vivo, a method for plasmid procedure for the detection of bacterial virulence gene

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Publication Types:

- Review
- Review, Tutorial

PMID: 7772291 [PubMed - indexed for MEDLINE]

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CAS Registry No.: 0 (DNA, Viral); 56-65-5 (Adenosine Triphosphate)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 3.1.- (Deoxyribonucleases)
Record Date Created: 19720930
Record Date Completed: 19720930

8/9/98
DIALOG(R)File 155:MEDLINE(R)

03270707 PMID: 4944854
Methylation in the phage carried **by escherichia coli 15T- (DNA methylation in phage psi).**
Medoff G
Virology (UNITED STATES) Jun 1971, 44 (3) p642-4, ISSN 0042-6822 Journal Code: 0110674
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Descriptors: *Coliphages; *DNA, Viral; *Escherichia coli; *Lysogeny; *Methylation; Adenine--analysis--AN; Centrifugation, Density Gradient; Cesium; Chloramphenicol--pharmacology--PD; Chlorides; Chromatography, Paper ; Colicins--analysis--AN; Coliphages--analysis--AN; DNA, Bacterial --analysis--AN; DNA, Bacterial--isolation and purification--IP; DNA, Viral --analysis--AN; DNA, Viral--isolation and purification--IP; Escherichia coli--analysis--AN; Escherichia coli--drug effects--DE; Escherichia coli --enzymology--EN; Escherichia coli--metabolism--ME; Genetics, Microbial; Methionine--metabolism--ME; Methyltransferases--metabolism--ME; Microscopy, Electron; Mitomycins--pharmacology--PD; **Mutation** ; Nitrosoguanidines; Spectrophotometry; Time Factors
CAS Registry No.: 0 (Chlorides); 0 (Colicins); 0 (DNA, Bacterial); 0 (DNA, Viral); 0 (Mitomycins); 0 (Nitrosoguanidines); 56-75-7 (Chloramphenicol); 63-68-3 (Methionine); 73-24-5 (Adenine); 7440-46-2 (Cesium)
Enzyme No.: EC 2.1.1. (Methyltransferases)
Record Date Created: 19720404
Record Date Completed: 19720404
?s s8 and heterol?
98 S8
46393 HETEROL?
S11 4 S8 AND HETEROL?
?t s11/9/all

11/9/1
DIALOG(R)File 155:MEDLINE(R)

13953474 PMID: 9654068
Methanol:coenzyme M methyltransferase from Methanosarcina barkeri--identification of the active-site histidine in the corrinoid-harboring subunit MtaC by site-directed mutagenesis .
Sauer K; Thauer R K
Max-Planck-Institut fur terrestrische Mikrobiologie and Laboratorium fur Mikrobiologie des Fachbereichs Biologie der Philipps-Universitat, Marburg, Germany.
European journal of biochemistry / FEBS (GERMANY) May 1 1998, 253 (3) p698-705, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The enzyme system catalyzing the formation of methyl-coenzyme M from methanol and coenzyme M in Methanosarcina barkeri is composed of the three

8/9/13
DIALOG(R)File 155:MEDLINE(R)

13209695 PMID: 8878670

DNA adenine methylase mutants **of Salmonella typhimurium and a novel dam-regulated locus.**

Torreblanca J; Casadesus J

Departamento de Genetica, Facultad de Biologia, Universidad de Sevilla, Spain.

Genetics (UNITED STATES) Sep 1996, 144 (1) p15-26, ISSN 0016-6731 Journal Code: 0374636

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Mutants of Salmonella typhimurium lacking DNA adenine methylase were isolated; they include **insertion** and deletion alleles. The dam locus maps at 75 min between cysG and aroB, similar to the Escherichia coli dam gene. Dam- **mutants** of S. typhimurium resemble those of E coli in the following phenotypes: (1) increased spontaneous **mutations** , (2) moderate SOS induction, (3) enhancement of duplication segregation, (4) inviability of dam recA and dam recB **mutants** , and (5) suppression of the inviability of the dam recA and dam recB combinations by **mutations** that eliminate

L9: Entry 1 of 41 File: USPT Sep 21, 2004

DOCUMENT-IDENTIFIER: US 6793927 B1
TITLE: Construction of Pasteurella haemolytica vaccines

Detailed Description Text (2):
It is a discovery of the present invention that P. haemolytica contains at least one restriction-modification system, called herein the PhaI system. Both the restriction endonuclease and the methyltransferase have been molecularly cloned. One such molecular clone (E. coli PhaIMtase) has been deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md., 20852, USA, on Dec. 2, 1993, under the terms of the Budapest Treaty as Accession No. ATCC 69500. A preliminary sequence of the methyltransferase gene has been determined The predicted amino acid sequence of the methyltransferase contains sequence motifs which ate consistent with an adenine-methylating specificity.

Detailed Description Text (5):
It has also been discovered by the present inventors, that a barrier to transformation of P. haemolytica can be overcome by treating DNA with a methylating enzyme, such as the PhaI methyltransferase. Such enzymes modify. DNA substrates such that endonucleases which recognize 5'-GATGC-3' or 5'-GCATC-3' sequences are inhibited in their ability to digest such modified substrates. Examples of such endonucleases are PhaI endonuclease and SfaNI endonuclease. While applicants do not wish to be bound by any particular hypothesis on the mechanism of action of such methyltransferase enzymes, it appears that the PhaI methyltransferase methylates specific adenine residues in DNA.

Detailed Description Text (51):
The possibility that a system similar to E. coli mcr, mrr, is active in P. haemolytica was investigated by passage of pPh.DELTA.roACm.sup.R pD80 through E. coli strain GM2163 previously transformed with the recombinant cosmid containing PhaI methyltransferase (Raleigh et al., Proc. Natl. Acad, Sci. 83:9070-9074 (1986)). Since strain GM2163 is dam-, the resultant DNA would only be modified at PhaI sites (Marinus et al., Mol. Gen. Genet. 192:288-289 (1983)). Efficiency of transformation with this DNA, however, was not substantially different than that using DNA obtained from PhaI Mtase which is dam-methylated (Table 1). It is possible a second restriction system, not readily detectable in cell extracts, is active in P. haemolytica A1. Genes have been described in Neisseria gonnorhea MS11 which encode for restriction enzymes which are expressed at levels too low to detect biochemically (Stein et al., J. Bact. 74:4899-4906 (1992).

L9: Entry 15 of 41 File: USPT Jan 2, 2001

US-PAT-NO: 6168918
DOCUMENT-IDENTIFIER: US 6168918 B1
**** See image for Certificate of Correction ****

TITLE: Method of detecting foreign DNA integrated in eukaryotic chromosomes

DATE-ISSUED: January 2, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Satishchandran; C.	Lansdale	PA		
Ciccarelli; Richard Benjamin	Pottstown	PA		
Pachuk; Catherine Julia	North Wales	PA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
American Home Products Corp.	Madison	NJ			02

APPL-NO: 08/ 594141 [PALM]
DATE FILED: January 31, 1996

INT-CL: [07] C12 Q 1/68, C12 P 19/34, C07 H 21/02

US-CL-ISSUED: 435/6; 435/912, 435/172.3, 435/240.2, 536/23.1
US-CL-CURRENT: 435/6; 435/912, 536/23.1

FIELD-OF-SEARCH: 435/6, 435/91.2, 435/172.3, 435/240.2, 935/24, 935/70, 935/77, 935/78, 536/23.1

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

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	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4683195	July 1987	Mullis et al.	435/6
☐	4683202	July 1987	Mullis	435/91
☐	4945050	July 1990	Sanford et al.	435/172.1
☐	4960707	October 1990	Lacks	435/320
☐	4965188	October 1990	Mullis et al.	435/6

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L9: Entry 5 of 41 File: USPT Sep 2, 2003

DOCUMENT-IDENTIFIER: US 6613749 B1
TITLE: Papovavirus pseudocapsids and use thereof for exogenous material transfer

Brief Summary Paragraph Table (1):
DISEASES CAUSED BY SINGLE GENE DEFECTS: CURRENT TARGETS FOR GENE THERAPY Disease Defective gene Immunodeficiency Adenosine deaminase Purine nucleosidc phosphorylase Hypercholesterolaemia LDL receptor Haemophilia Factor IX Factor VIII Gaucher's disease Glucocerebrosidase Mucopolysaccharidosis .beta.-glucuronidase Emphysema .alpha.-antitrypsin Cystic fibrosis Cystic fibrosis transmembrane regulator Phenylketonuria Phenylalanine hydroxylase Hyperammonaemia Ornithine transcarbamylase Citrullinaemia Arginosuccinate synthetase Muscular dystrophy Dystrophin Thalassaemia .beta.-globin Sickle cell anaemia .beta.-globin Leukocyte adhesion deficiency CD-18

Detailed Description Text (4):
Escherichia coli, strain DH5, was used for the propagation of transfer vectors and recombinant plasmids. Where a digestion with the BclI enzyme was used, plasmids were propagated in E. coli JM110 strain (a dam dcm mutant derivative of JM 101) (Yanish-Perron et al., 1985, Gene 33: 103-119).

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L9: Entry 18 of 41 File: USPT Apr 4, 2000

DOCUMENT-IDENTIFIER: US 6046173 A
TITLE: Polyoma virus pseudocapsids and method to deliver material into cell

Brief Summary Paragraph Table (1):

DISEASE CAUSED BY SINGLE GENE DEFECTS: CURRENT TARGETS FOR GENE THERAPY Disease Defective gene

Immunodeficiency Adenosine deaminase Purine nucleoside phophorylase Hypercholesterolaemia LDL receptor Haemophilia Factor IX Factor VIII Gaucher's disease Glucocerebrosidase Mucopolysaccharidosis .beta.-glucuronidase Emphysema .alpha.-antitrypsin Cystic fibrosis Cystic fibrosis transmembrane regulator Phenylketonuria Phenylalanine hydroxylase Hyperammonaemia Orinthine transcarbamylase Citrullinaemia Arginosuccinate synthetase Muscular dystrophy Dystrophin Thalassaemia .beta.-globin Sickle cell anaemia .beta.-globin Leukocyte adhesion deficiency CD-18

Detailed Description Text (3):
Escherichia coli, strain DH5, was used for the propagation of transfer vectors and recombinant plasmids. Where a digestion with the BclI enzyme was used, plasmids were propagated in E. coli JM110 strain (a dam dem mutant derivative of JM101) (Yanish-Perron et al., 1985, Gene 33: 103-119).

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L26: Entry 51 of 74 File: USPT Jul 2, 2002

US-PAT-NO: 6413751
DOCUMENT-IDENTIFIER: US 6413751 B1

TITLE: DNA adenine methyltransferases and uses thereof

DATE-ISSUED: July 2, 2002

WO98/12206 March 26 1998 (29)

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Benkovic; Stephen J.	State College	PA		
Berdis; Anthony	Shaker Heights	OH		
Lee; Irene	Shaker Heights	OH		
Shapiro; Lucy	Stanford	CA		
Wright; Rachel	Menlo Park	CA		
Stephens; Craig	Mountain View	CA		
Kahng; Lyn Sue	Mountain View	CA		

US-CL-CURRENT: 435/193; 536/23.2

CLAIMS:

What is claimed is:

1. An isolated DNA adenine methyltransferase wherein said methyltransferase has has an amino acid sequence as set forth in SEQ ID No. 6.

2. An isolated nucleic acid that encodes a Helicobacter pylori DNA methyltansferase having an amino acid sequence comprising.

3. An isolated nucleic acid of claim 2, wherein the nucleic acid comprises SEQ ID NO:7.

4. A nucleic acid of claim 2 contained in a genetically engineered cell.

5. An isolated DNA adenine methyltransferase having an amino acid sequence as set forth in SEQ ID NO: 8.

US-PAT-NO: 6413751
DOCUMENT-IDENTIFIER: US 6413751 B1

TITLE: DNA adenine methyltransferases and uses thereof

DATE-ISSUED: July 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Benkovic; Stephen J.	State College	PA		
Berdis; Anthony	Shaker Heights	OH		
Lee; Irene	Shaker Heights	OH		
Shapiro; Lucy	Stanford	CA		
Wright; Rachel	Menlo Park	CA		
Stephens; Craig	Mountain View	CA		
Kahng; Lyn Sue	Mountain View	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The Board of Trustees of the Leland Stanford Jr. University	Stanford	CA			02
The Penn State Research Foundation	University Park	PA			02

APPL-NO: 09/ 269137 [PALM]
DATE FILED: July 19, 1999

PARENT-CASE:
CROSS REFERENCES TO RELATED APPLICATIONS The present application claims priority from U.S. provisional Application No. 60/020,089, filed Sep. 19, 1996.

PCT-DATA:

APPL-NO	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/US97/16593	September 17, 1997	WO98/12206	Mar 26, 1998	Jul 19, 1999	Jul 19, 1999

INT-CL: [07] C12 N 9/10, C07 H 21/04

US-CL-ISSUED: 435/193; 536/23.2
US-CL-CURRENT: 435/193; 536/23.2

FIELD-OF-SEARCH: 435/193, 536/23.2

PRIOR-ART-DISCLOSED:

OTHER

DOCUMENT-IDENTIFIER: US 6395965 B1
TITLE: Plant containing a gene construct comprising a chlorella virus promoter and a lac operator

Other Reference Publication (20):
Xia et al. "Adenine DNA methyltransferase M. CviR1 expression accelerates apoptosis in baculovirus-infected insect cells," Academic Press, New York, NY, Virology, 196: 817-824 (1993).

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2 plasmids w dam mutant heterologous DNA but not from pathogen

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L9: Entry 38 of 41 File: USPT Apr 13, 1993

DOCUMENT-IDENTIFIER: US 5202256 A
TITLE: Bioadhesive precursor protein expression vectors

Detailed Description Text (137):
An in-frame gene fusion between the trpB portion of pGX2346, and the bioadhesive precursor protein cDNA of pGX2368 is constructed in the following manner: A BclI endonuclease recognition site was first placed at the translation stop codon of pGX2368 by changing two bases as indicated in FIG. 1 using oligonucleotide-directed mutagenesis (Zoller, M. J. and M. Smith, Methods Enzymol., 100:457-500, 1983) to create plasmid pGX2380. Both plasmids pGX2380 and pGX2346 are grown for DNA preparation in an E. coli host that contains the dam mutation (defective in DNA adenine methylase) so that they could be digested with BclI. The non-methylated pGX2346 DNA is cut with NotI and the pGX2380 DNA is cut with XbaI. Then both DNAs are treated with E. coli DNA polymerase (Klenow fragment) to fill in the 5' single-stranded DNA overhangs. The DNAs are then ligated at high DNA concentration (approximately 2 ug of each DNA in 20 ul) with T4 ligase. The ligation product is cut with BclI then ligated again at low DNA concentration (approximately 1 ug total DNA in 150 ul volume) and used to transform E. coli GX3015. A transformant with the desired construction (see FIG. 6) is designated pGX2383. GX3015 cells with plasmid pGX2383 produce a bioadhesive precursor protein of approximately 24,000 M.W. upon induction of the hybrid lambda promoter by a shift of growth temperature from 32.degree. to 37.degree. C.

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L9: Entry 36 of 41 File: USPT Sep 19, 1995

DOCUMENT-IDENTIFIER: US 5451519 A
TITLE: Cloning restriction endonuclease genes by modulating methyltransferase activity

Detailed Description Text (18):
For instance, preferred hosts do not express an endogenous methyltransferase activity that adversely affects cellular growth in the presence of low cellular concentrations of the methyl donor co-factor. Thus, for example, preferred E. coli hosts are dam. E. coli cells of this genotype do not express the E. coli DNA adenine methyltransferase, which regulates DNA replication. This enzyme methylates newly replicated DNA. Low cellular concentrations of the methyl donor co-factor S-adenosyl-L-methionine inhibit the activity of the enzyme leading to the production in the cell of hemimethylated DNA in the host. Hemimethylated DNA is not well replicated by E. coli. Therefore, low cellular concentration of S-adenosyl-L-methionine inhibits cell growth, which can interfere with some assays useful in the invention to detect the presence of the cloned restriction endonuclease. Thus, E. coli hosts for use in the invention are preferably dam. The dam system and E. coli mutants in this system are described in Landoulsi et al., Cell 63: 1053 (1990), for instance, herein incorporated by reference in its entirety.

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